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Possible association between early formula and reduced risk of cow's milk allergy: The Japan Environment and Children's Study

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Abstract

Background: Despite evidence for the protective effects of early regular exposure to peanut and egg proteins against allergies, the optimal timing of cow's milk (CM) protein exposure is unknown.

Objective: We aimed to determine when during the first year of life CM-based formula consumption becomes associated with lower CM allergy (CMA) risk.

Methods: We used the data set of the Japan Environment and Children's Study (JECS), a nationwide birth cohort involving over 100 000 mother-child pairs. CMA was defined as an allergic reaction to a CM product in an individual not consuming CM products at the time of evaluation, combined with physician-diagnosed food allergy. For each exposure, we identified when formula milk was commenced, and its consumption status during 0-3, 3-6 and 6-12 months old.

Results: The prevalence of CMA was 0.23% and 1.03% at 6 and 12 months old, respectively. Multivariable regression analyses revealed that introducing regular consumption of formula within the first 3 months of age was associated with lower risk of CMA at 12 months. Regular consumption at 3-6 months was strongly associated with a reduction in 12-month CMA (adjusted relative risks [95% confidence intervals]: 0.22 [0.12-0.35]), whereas no association was observed at 0-3 months (1.07 [0.90-1.27]).

Conclusion and Clinical Relevance: Regular exposure to formula milk at age 3 months or older is associated with lower CMA at 12 months old, suggesting that the effect of very early CM exposure on CMA may disappear if the exposure is brief. At present, however, the results of this observational study should not be used for formula recommendation and randomized controlled trials are required to confirm this association.

Junichiro Tezuka and Masafumi Sanefuji are contributed equally to the work.

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KEYWORDS
epidemiology, food allergy, pediatrics

1 | INTRODUCTION

The influence of diets during pregnancy and infancy on child's risk of allergic disease has been of great interest but largely remains inconclusive.[1] Among a variety of allergenic diets, cow's milk (CM) is often the first foreign protein to which infants are exposed in the form of infant formula, and cow's milk allergy (CMA) is among the most common food allergies in childhood. Although breastfeeding has many benefits and is thus recommended for all infants, formula feeding can be introduced concurrently or exclusively for various reasons (eg insufficient breastmilk, mother's work or disease, etc). Contrary to the accumulating evidence that early introduction of peanut and hen's egg proteins reduces the risk of allergy to these foods,[2,3] it remains controversial whether early introduction of CM protein is related to a reduction in CMA; whereas some previous studies have shown a protective effect,[4-7] others have not.[8,9] These conflicting results could be attributable not only to variations in study design and ethnicity among studies, but also to the relatively small numbers of participants included. Furthermore, a few studies have suggested a higher risk of CMA in infants who consume formula milk from birth but discontinue it in early infancy.[6,7] However, most previous studies have focused only on the time when infants start consuming formula, and not on whether they continue or discontinue consumption.

The goal of the present study was to explore the optimal time window for formula consumption that may be associated with a reduction in CMA, using data from the Japan Environment and Children's Study (JECS), a nationwide government-funded birth cohort study. This study includes more than 100 000 children across Japan and aims to determine environmental factors affecting children's health without a specific focus on allergic diseases, such that the sample is representative of the general population. In this data set, some mothers breastfed exclusively after delivery and later started formula milk concurrently or exclusively whereas others used formula, for example, to temporally supplement insufficient breastmilk in early life of the child and discontinued it subsequently. Such divergent feeding patterns enabled the analyses on period-specific influence of formula on CMA development.

2 | METHODS

2.1 | Design

The JECS is a nationwide, multi-centre, prospective birth cohort study funded by the Ministry of the Environment, Japan. The study design details have been described previously.[10,11] Briefly, the JECS registered 103 099 pregnancies, including multiple conceptions, between January 2011 and March 2014 in 15 regional centres covering a wide geographical area of Japan. In addition, 51 909 male partners were registered. During pregnancy, information was obtained twice, during the first and second/third trimesters, from the pregnant women, and once from the partner, using self-administered questionnaires. Detailed information regarding the mother and child was obtained from medical records during the first trimester, at delivery, and when the child was 1 month-old. After delivery, information was collected at the age of 1 month, and then every 6 months following the birth (at 6, 12 and 18 months, etc) *via* self-reported questionnaires completed by the parents. The JECS protocol was reviewed and approved by the Ministry of Environment's Institutional Review Board for Epidemiologic Studies (#100910001) and by the ethics committees of all participating institutions (#2019-070). The JECS was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all parents for their participation and for that of their children.

2.2 | Participants

In this study, we used a fixed data set "jecs-an-20180131," which was released in March 2018. The data set contains all available data for 104 065 fetuses, which are linked to the respective maternal and paternal data that was collected until the child was 12 months old. A total of 88 538 live-born children for whom information on sex and birthweight had been recorded and for whom data were available at 1, 6 and 12 months old were selected as participants (Figure 1). Of these children, we excluded those whose reply was delayed (>2 months after questionnaire delivery) at 6 or 12 months old and those who had missing information on feeding habits during the first year of life or on consumption status of dairy food at 12 months old. Among the remaining 82 726 children who had confirmed data on feeding and dairy food, we further excluded those who used specialized formula for CMA, which is available as extensively or partially hydrolysed, amino acid or soy-based formula in Japan, as queried at 6 months old. After excluding all these 8130 children, data for 80 408 children remained for analysis.

2.3 | Exposure

The main exposure factor was regular consumption of infant formula milk. Mothers were asked to provide the monthly status of their child using questionnaires at the age of 1, 6 and 12 months. This information included whether the child was breastfed, used formula, or used a mixture of both, and allowed the monthly feeding habits of the children enrolled in this study to be determined. On the basis of this information, we determined whether a child started consuming formula milk early, which was defined as its introduction within the first 3 months of life. However, this

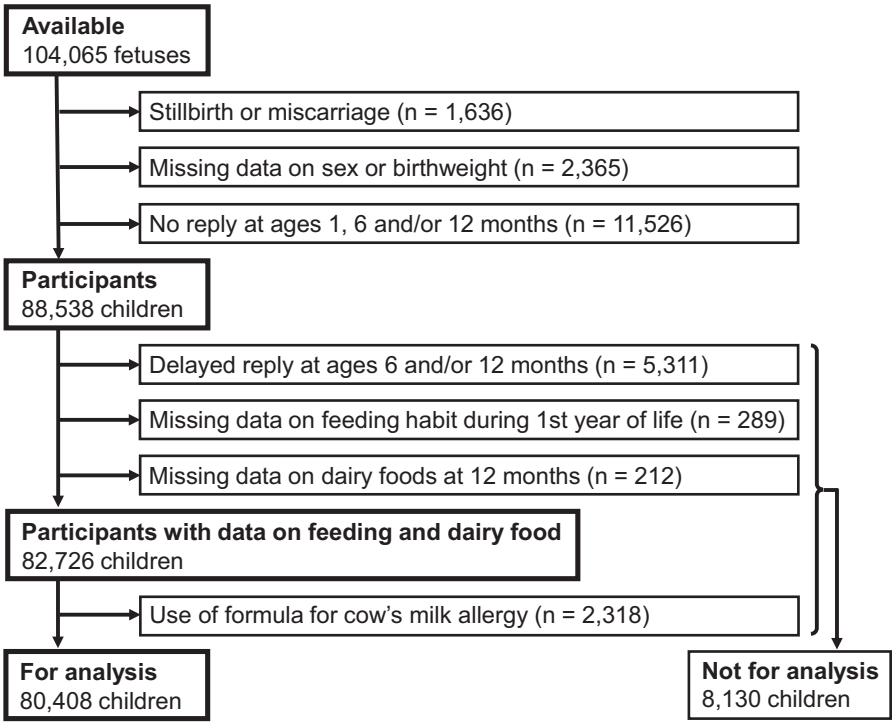

FIGURE 1 Flow chart for participant selection

dichotomous classification of early *vs*. late introduction did not discriminate whether or when the child discontinued formula milk thereafter. To identify the critical periods associated with the development of CMA, we also determined the patterns of formula feeding during the first year of life for each infant, irrespective of concurrent consumption of breast milk. Because the monthly data collected produce an exponential number of consumption patterns (2^{12} patterns), we categorized consumption into 3 periods: period-1 was 0-3 months old; period-2, 3-6 months; and period-3, 6-12 months. In each period, an infant was defined as consuming substantially formula milk if he or she received formula milk over half of the period (for 2 or 3 months during the 3-month period in period-1 and −2; for 4, 5 or 6 months during the 6-month period in period-3). For example, if an infant consumed formula milk during its first 2 months of age but not during the third month, he or she was regarded as consuming substantially formula milk in period-1. This creation of the three periods generated a more limited number of patterns ($2^3 = 8$ patterns), which simplified subsequent analyses. To validate the appropriateness of this definition of the "approximated period," we also analyzed "exact period," extracting data for infants who consumed formula milk for the full months during a period and for those who did not consume any formula milk in that period. As an additional exposure factor, breast milk was similarly categorized.

2.4 | Outcome

The primary outcome was the development of CMA prior to 6 or 12 months old. Inclusion criteria for CMA were (a) parent-reported allergic reactions of the child to CM products such as formula milk or dairy food, (b) no consumption of CM products at the evaluation times of 6 and 12 months, and (c) food allergy diagnosed by a community physician who were not involved in the JECS study. In the 6-month questionnaire, a reaction was defined as the appearance of any symptoms within 1 hour of the ingestion of formula or dairy products, but in the questionnaire completed at 12 months, the time window for the appearance of symptoms was set to 3 hours after ingestion. The "3 hours" time window was less likely to omit the symptoms that were recollected when filling in the questionnaires. The "1 hour" time window was adequate for infants before 6 months old because they could be fed frequently (eg every 2 hours) in early infancy. The symptoms included urticarial or itchy skin, face or lip oedema, a pale face, loss of consciousness, cough, wheezing, vomiting and diarrhoea. Diagnoses of food allergy were made at the age of 12 months or younger, and an infant was regarded as having CMA when he or she fulfilled all three criteria.

2.5 | Statistical analysis

To evaluate the relationship between formula feeding and CMA, we conducted logistic regression analyses, which were adjusted for the following covariates: i) sex; ii) any maternal allergic disease; iii) maternal smoking during pregnancy, confirmed in the first trimester; iv) maternal education level (junior high school, high school, or university or graduate school); v) annual family income; vi) early eczema, occurring within the first 3 months of life and defined as an intermittent itchy rash over a period of ≥2 months; and vii) living with older siblings. Statistical analyses were performed using R software, version 3.5.0. Firth's bias reduction method was employed when separation occurred in logistic regression analysis,

TABLE 1 Baseline characteristics of the participants (n = 88 538)

	For analysis (n = 80 408)	[Missing]	Not for analysis (n = 8130)	[Missing]	Effect size[a] (*vs.* for analysis)	Formula for CMA (n = 2318)	[Missing]	Effect size[a] (*vs.* for analysis)
Males	41 125 (51.1%)	0	4269 (52.5%)	0	0.01	1261 (54.4%)	0	0.01
Gestational age (week)	39.2 ± 1.5	0	39.0 ± 1.9	0	0.03	39.2 ± 1.5	0	0.00
Birth weight (g)	3016 ± 419	0	2993 ± 467	0	0.02	3041 ± 421	0	0.01
Maternal age (years)	31.4 ± 4.9	3	31.2 ± 5.2	1	0.01	31.6 ± 5.0	0	0.01
Maternal allergic diseases	43 825 (54.7%)	353	4661 (57.9%)	74	0.02	1493 (64.7%)	9	0.03
Maternal smoking during pregnancy	12 732 (16.0%)	848	1597 (20.0%)	162	0.03	359 (15.7%)	24	0.00
Maternal education								
Junior high school	3137 (3.9%)	739	420 (5.3%)	170	0.03	64 (2.8%)	20	0.01
High school	58 315 (73.2%)		5990 (75.3%)			1718 (74.8%)		
University/graduate school	18 217 (22.9%)		1550 (19.5%)			516 (22.5%)		
Family income								
Low (<4 000 000 JPY)	29 229 (39.1%)	5599	2969 (40.4%)	780	0.01	810 (37.7%)	171	0.01
Middle (4 000 000-5 999 999 JPY)	25 094 (33.5%)		2417 (32.9%)			716 (33.3%)		
High (≥6 000 000 JPY)	20 486 (27.4%)		1964 (26.7%)			621 (28.9%)		
Early eczema	19 059 (24.1%)	1471	1818 (22.9%)	189	0.01	696 (30.6%)	46	0.02
Older siblings	43 411 (54.2%)	353	4477 (55.6%)	74	0.01	1034 (44.8%)	9	0.03
Any symptoms after CM products								
By 6 months old	720 (0.9%)	0	321 (3.9%)	0	0.08	195 (8.4%)	0	0.12
By 12 months old	3016 (3.8%)	0	509 (6.3%)	0	0.04	247 (10.7%)	0	0.06
Formula introduction by 3 months old	48 820 (60.7%)	0	5599 (71.6%)	315	0.06	2040 (88.0%)	0	0.09
Introduction of dairy foods								
By 6 months old	3561 (4.5%)	516	376 (4.7%)	151	0.00	78 (3.4%)	18	0.01
By 12 months old	73 385 (91.9%)	516	6699 (84.0%)	151	0.08	1939 (84.3%)	18	0.05
Prevalence of CMA								
At 6 months old	182 (0.2%)	0	48 (0.6%)	0	0.02	16 (0.7%)	0	0.02
At 12 months old	824 (1.0%)	0	87 (1.1%)	4	0.00	24 (1.0%)	0	0.00

Abbreviations: CM, cow's milk; CMA, cow's milk allergy; JPY, Japanese yen.

[a]Effect sizes are calculated as *phi*/Cramer's *V* and *r* using chi-square and Student's *t* tests for categorical and numerical variables, respectively.

Period-1 (0–3m)	Period-2 (3–6m)	Period-3 (6–12m)	Pattern	Number		6m-CMA, No. (%)		12m-CMA, No. (%)	
92.1%	60.8%	35.4%	1	24,721	27,687	0 (0)	1 (0.00)	1 (0.00)	10 (0.04)
94.2%	89.2%		2	2,966		1 (0.03)		9 (0.30)	
97.7%		74.1%	3	1,313	10,167	3 (0.23)	34 (0.33)	0 (0)	178 (1.75)
99.5%			4	8,854		31 (0.35)		178 (2.01)	
	69.7%	35.2%	5	5,392	6,124	0 (0)	0 (0)	1 (0.02)	6 (0.10)
	91.9%		6	732		0 (0)		5 (0.68)	
		62.3%	7	4,779	36,430	20 (0.42)	147 (0.40)	0 (0)	630 (1.73)
			8	31,651		127 (0.40)		630 (1.99)	
			total	80,408		182 (0.23)		824 (1.02)	

FIGURE 2 Patterns of formula feeding and the prevalence of cow's milk allergy. Grey and white boxes denote consumption and non-consumption, respectively, of formula milk during each period. The number in the grey boxes represents the percentage of concurrent use of breastmilk in formula-fed children. For each pattern, the prevalence of children with cow's milk allergy (CMA) at 6 and 12 months old is provided. The prevalence in greyed areas is zero or nearly zero as expected from the definition of CMA, which required no consumption of CM products including formula at the evaluation times

using "logistf" version 1.23 in the R package. We reported adjusted relative risks (aRRs) with 95% confidence intervals (CIs) and *P*-values.[12]

3 | RESULTS

3.1 | Study population

The baseline characteristics of the 80 408 children included in our analysis are shown in Table 1. Allergic disease was recorded in 54.7% of mothers and early eczema was observed in 24.1% of children. Any symptoms after ingestion of CM products were observed by 6 months in 720 (0.90%) but CMA actually developed in 182 (0.23%) children by that time. Among the CMA children, 139 (76.4%) children were reported as having reaction to formula; 31(17.0%) to dairy food; 12 (6.6%) to both. By 12 months old, any symptoms after CM ingestion were observed in 3016 (3.75%) children whereas CMA eventually developed in 824 (1.03%) children: 198 (24.0%) reacted to formula; 450 (54.6%) to dairy food; 176 (21.4%) to both.

The majority of characteristics of the 8130 children who were not analyzed were similar to those of the children for analysis (effect sizes ≤ 0.03). However, there were some differences in occurrence of symptoms after CM products and introduction of formula and dairy foods between these children and those for main analysis. The 2318 children who used specialized formula at 6 months old had a tendency to have early eczema and maternal allergic diseases than the children for analysis. By 6 months of age, they were reported to show symptoms after CM ingestion much more highly than the children for analysis (8.4% *vs*. 0.9%) whereas the actual prevalence of CMA was not much different at that time (0.69% *vs*. 0.23%). In this group, formula was more likely to have been introduced by 3 months old (88.0% *vs*. 60.7%) although the information was lacking whether the formula was specialized or not at that time, and dairy food was less likely to have been introduced by 12 months old (84.3% *vs*. 91.9%).

3.2 | Early introduction of formula milk

We evaluated the relationship between early introduction of formula feeding and CMA using multivariable regression models. Early introduction of formula by 3 months old was reported in 48 820 (60.7%) children, and was associated with a reduction in CMA at the age of 6 and 12 months (aRR [95% CI]: 0.42 [0.30-0.57] and 0.44 [0.38-0.51], respectively). When dairy food was additionally included as a CM protein source in this analyses, only at most 18 children were added to the 48 820 children and therefore the results were virtually identical (0.43 [0.31-0.59] and 0.45 [0.38-0.52], respectively). These models also revealed that higher risks of CMA were associated with early eczema (5.60 [4.07-7.79] and 4.78 [4.13-5.54] for CMA at 6 and 12 months, respectively). Log-likelihood ratio tests revealed no significant differences in the models with and without the interaction terms between early eczema and early formula introduction (difference in deviance [*P*-value]: .01 [.90] and 0.42 [.51] for CMA at 6 and 12 months, respectively).

3.3 | Periods of formula consumption

We explored which periods of time were specifically associated with a risk of CMA. To provide a comprehensive overview, we classified the participants into 8 patterns according to whether they consumed substantially formula or not, irrespective of concurrent consumption of breast milk, in the three periods (periods-1-3), and calculated the prevalence of CMA for each pattern (Figure 2). Note that the prevalence of CMA at 6 months old was expected to be zero or nearly zero when the child consumed formula during period-2 (patterns 1, 2, 5

and 6) because a child was defined as having no CMA when he or she consumed CM products including formula at the evaluation times. Similar relationships were observed between the prevalence of CMA at 12 months and formula consumption during period-3 (patterns 1, 3, 5 and 7). Thus, we do not mention such self-explanatory combinations in the following analyses.

During period-1, most of the children who consumed formula milk were concomitantly breastfed, that is, mixed-fed. At 6 months old, the prevalence of CMA was comparable between children who consumed formula during period-1 (0.33% totalled for patterns 3 and 4) and children who did not during that period (0.40% for patterns 7 and 8). At 12 months old, overall, the prevalence of CMA was low when a child consumed formula during period-2 (0.04% for patterns 1 and 2, and 0.10% for patterns 5 and 6) compared with no formula consumption (1.75% for patterns 3 and 4, and 1.73% for patterns 7 and 8). Between patterns 2 and 4, for example, the statuses of formula consumption were different in period-2 but identical in the other periods (period-1 and −3) while the prevalence in pattern 2 (0.30%) was lower than those in pattern 4 (2.01%). This was the case for patterns 6 (0.68%) *vs*. 8 (1.99%). These results of the "approximated period" were similar to those of the "exact period" (Figure S1), in which, however, the numbers of children included were considerably reduced.

The RRs for CMA at 6 and 12 months old with respect to each period were estimated using multivariable regression models adjusted for key covariates (Table 2). To determine which periods more directly influenced the prevalence of CMA, we included all periods as well as potential confounding factors together in the models. Using such models permitted us to determine the impact of each period alone, without the influence of other periods. The analysis

TABLE 2 CMA in association with periods of formula consumption

a. All children for analysis				
	6 m-CMA (n = 72 727)		**12 m-CMA (n = 72 727)**	
Formula consumption	**aRR (95% CI)**	***P*-value**	**aRR (95% CI)**	***P*-value**
Period-1 (0-3 m)	0.92 (0.61-1.34)	.67	1.07 (0.90-1.27)	.45
Period-2 (3-6 m)	0.01 (0.00-0.04)[a]	<.001	**0.22 (0.12-0.35)**	**<.001**
Period-3 (6-12 m)	–	–	0.01 (0.00-0.03)[a]	<.001
b. Exclusion of children who started dairy food by the evaluation times of CMA				
	6 m-CMA (n = 69 144)		**12 m-CMA (n = 5838)**	
Formula consumption	**aRR (95% CI)**	***P*-value**	**aRR (95% CI)**	***P*-value**
Period-1 (0-3 m)	0.94 (0.61-1.39)	.75	**1.47 (1.16-1.83)**	**.001**
Period-2 (3-6 m)	0.01 (0.00-0.05)[a]	<.001	**0.21 (0.09-0.42)**	**<.001**
Period-3 (6-12 m)	–	–	0.01 (0.00-0.04)[a]	<.001
c. Exclusion of children who already had CMA at 6 months				
	6 m-CMA		**12 m-CMA (n = 72 561)**	
Formula consumption	**aRR (95% CI)**	***P*-value**	**aRR (95% CI)**	***P*-value**
Period-1 (0-3 m)	–	–	1.08 (0.90-1.29)	.41
Period-2 (3-6 m)	–	–	**0.24 (0.14-0.40)**	**<.001**
Period-3 (6-12 m)	–	–	0.01 (0.00-0.03)[a]	<.001
d. Children who used specialized formula for CMA				
	6 m-CMA (n = 2085)		**12 m-CMA (n = 2085)**	
Formula consumption	**aRR (95% CI)**	***P*-value**	**aRR (95% CI)[b]**	***P*-value**
Period-1 (0-3 m)	**0.19 (0.06-0.55)**	**.003**	**0.24 (0.09-0.58)**	**.002**
Period-2 (3-6 m)	0.04 (0.00-0.23)[a]	.003	0.94 (0.31-2.42)	.91
Period-3 (6-12 m)	–	–	0.01 (0.00-0.11)[a]	<.001

Note: All the periods of formula consumption and the following covariates were included together in the model: sex, maternal allergic diseases, smoking and education, family income, early eczema and older siblings. Bold text represents statistical significance ($P < .05$).

Abbreviations: aRR, adjusted relative risk; CI, confidence interval; CMA, cow's milk allergy.

[a]The result was very low as expected from the definition of CMA, which required no consumption of CM products including formula at the evaluation time.

[b]Using Firth's bias reduction method to resolve the issue of separation in regression analysis.

TABLE 3 CMA in association with the periods of formula and breastmilk

	6 m-CMA (n = 72 727)		12 m-CMA (n = 72 727)	
Consumption	**aRR (95% CI)**[b]	***P*-value**	**aRR (95% CI)**	***P*-value**
Period-1 (0-3 m)				
Formula	0.96 (0.61-1.42)	.84	1.07 (0.89-1.30)	.48
Breastmilk	0.80 (0.07-24.0)	.89	0.79 (0.17-5.25)	.78
Period-2 (3-6 m)				
Formula	0.02 (0.00-0.10)[a]	<.001	**0.25 (0.12-0.48)**	**<.001**
Breastmilk	1.24 (0.17-75.20)	.88	1.22 (0.29-9.81)	.82
Period-3 (6-12 m)				
Formula	–	–	0.01 (0.00-0.03)[a]	<.001
Breastmilk	–	–	1.34 (0.29-6.18)	.67

Note: The interaction terms (period-1 of formula × period-1 of breastmilk, period-2 of formula × period-2 of breastmilk for 6 m-CMA; period-3 of formula × period-3 of breastmilk were further added for 12 m-CMA) and the following covariates were included in the model: sex, maternal allergic diseases, smoking and education, family income, early eczema and older siblings. Bold text represents statistical significance ($P < .05$).

Abbreviations: aRR, adjusted relative risk; CI, confidence interval; CMA, cow's milk allergy.

[a]The results were very low as expected from the definition of CMA.

[b]Using Firth's bias reduction method.

for CMA at 6 months old revealed that formula consumption during period-1 was not associated with the prevalence of CMA (0.92 [0.61-1.34], Table 2a). The analysis for CMA at 12 months showed that formula consumption during periods-2 were strongly associated with a lower prevalence of CMA (0.22 [0.12-0.35]) whereas consumption during period-1 showed no association (1.07 [0.90-1.27]). The results of analyses using the "approximated period" were similar to those obtained using the "exact period" (Table S1).

However, the results shown above may be influenced by another source of CM protein, dairy foods such as yogurt and cheese, especially late during the first year of life. During the first year of age, unfortunately, the JECS collected only the commencement time of dairy food and not the detailed consumption status. To eliminate the influence, we performed an additional analysis after excluding 3561 and 73 385 children who had commenced dairy foods by 6 and 12 months old, respectively, and then found a similar result (Table 2b). Further, there would be a concern about the relationship between formula consumption and CMA, both of which might be interdependent. After a child had an allergy to formula at a certain point (eg at 4 months of age), the mother would likely avoid formula feeding for a long time (eg even at 12 months of age). This avoidance had the potential to produce a superficial association between no formula consumption and higher 12-month CMA. To gain better insight into the issue, we excluded the 182 children who developed CMA by 6 months of age and conducted the analysis on the remaining children. If a child who had experienced regular consumption before 6 months old (ie during period-1 or −2) showed a reduced risk of CMA that developed after 6 months, early formula consumption was suggested to be an influential factor for later development of CMA. This analysis yielded a concordant result (Table 2c), too.

When the analyses were conducted on the 2318 children who used specialized formula for CMA, the association with 12-month CMA was observed in period-1 (0.24 [0.09-0.58]) but not in period-2 (0.94 [0.31-2.42], Table 2d). However, these results should be cautiously interpreted because information on the formula used in this group during the other periods except at 6 months old was not obtained. A sensitivity analysis that included both of the children for main analysis and those who used specialized formula yielded similar results (Table S1).

To assess whether breast milk might modify the risk of CMA, we added the consumption periods of breast milk as well as interaction terms of each period between formula milk and breast milk to the statistical models and conducted regression analyses (Table 3). The periods of formula milk had similar associations with the risk of CMA at 12 months old while those of breast milk had no associations. A log-likelihood ratio test revealed no significant difference in these models with and without the interaction terms (difference in deviance: 4.01, *P*-value: .26).

4 | DISCUSSION

Using the data set from a nationwide survey of the general Japanese population, we have demonstrated that early introduction of formula milk feeding is associated with a lower risk of CMA during the first year of life. Furthermore, we have shown for the first time that regular consumption of formula at the age of 3 months or older is more closely associated with a reduction in CMA than consumption during the earlier periods. These findings suggest that the effects of very early introduction of formula on CMA can diminish when formula consumption is brief.

To the best of our knowledge, this is the largest birth cohort study of the relationship between formula consumption and the development of CMA during the first year of life. This large data set enabled the calculation of CMA prevalence for 8 patterns of formula feeding and the identification of the critical time period influencing CMA

development. Monthly information regarding formula consumption was obtained longitudinally using successive questionnaires that were completed when the children were 1, 6 and 12 months old, minimizing the risk of recall bias. The feeding information provided was used to determine the feeding pattern, which described when the child commenced, discontinued and occasionally re-started regular consumption of formula milk. This detailed profiling revealed that the association between exposure to CM protein and CMA depends on the specific period during which formula consumption occurs.

Several studies have shown an association between a lower risk of CMA and early introduction of CM-based formula milk. A lower risk of CMA has been associated with regular consumption of formula during the first 14 days,[4] and the first 1,[5] 2,[6] and 3 months[7] of life. However, these studies provided little information regarding subsequent exposure. Our study has provided detailed information regarding the status of formula feeding, month-by-month, and has provided evidence that the CMA risk is not solely determined by when formula milk is introduced.

The present study appears to be at least partially consistent with the dual-allergen exposure hypothesis.[13,14] This hypothesis states that early cutaneous exposure to food protein through a disrupted skin barrier leads to allergic sensitization, whereas early oral exposure to food allergens induce tolerance. Cutaneous sensitization to CM was not shown in the present study, because no interaction was demonstrated between eczema and early introduction of formula milk. Conversely, the induction of oral tolerance in infancy is strongly supported by our finding of the close association between formula consumption and reduced risk of CMA. One hypothesis proposes that tolerance to food allergens is driven by regular exposure to these proteins during a critical time window, considered to be between 3 and 7 months of life.[15] This hypothesis may explain the stronger association between reduced risk of CMA and formula consumption at 3 months or older than at 0-3 months in our study. CM protein is often introduced from birth as formula milk, whereas other allergenic proteins such as egg and peanut are usually started at the age of 4 months or older as solid foods. Our analyses of formula milk consumption suggest that the critical window for allergy begins at 3 months old.

Recently, the Enquiring about Tolerance (EAT) study reported no association between CMA and the early introduction of CM protein.[9] This lack of the association may be attributable to the relatively small number of participants in this study. Furthermore, the parents of infants in the early introduction group (at 3 months old) were recommended to feed their infants 4 g of CM protein per week as yogurt. The actual amount fed, which was lower than recommended because of compliance issues, is much lower than the estimated amount of CM protein (79 g per week) administered in formula milk (median of 750 mL/day for infants fed exclusively with formula or a mixture, recorded in a questionnaire completed at 6 months) in our study. Therefore, in the EAT study, the amount of CM protein fed might have been too small to induce immune tolerance.

In the 82 726 JECS children with data on feeding and dairy food, 2318 (2.8%) children used specialized formula for CMA at 6 months old. Among them, however, only 24 (1.04%) were eventually defined as having CMA by 12 months of age. The reason for the use of specialized formula was not asked in the questionnaire. Because this group was more likely to have early eczema and maternal allergic diseases than the children for main analysis, specialized formula might be used for prevention of CMA. Further, any symptoms after CM ingestion were more frequently reported in this group. Some parents would judge these symptoms due to food allergy to CM protein derived from maternal diet *via* breast milk or from infant formula, and then feed them with specialized formula. Such parent's self-judgement might be reflected by the earlier introduction of formula, which was probably specialized formula for CMA, and slower introduction of dairy food. As shown in Table 2d, interestingly, the association between period-2 (3-6 months of age) of formula and CMA at 12 months was not observed in these children. Because it was unknown whether the specialized formula was used consistently through to 6 months of age or switched from regular formula in our data set, the result was inconclusive but might support the finding that specialized formula has not been proven to prevent CMA.[16,17] As there is insufficient evidence for allergy-preventing effects of maternal or infant CM restriction during breastfeeding,[18-20] we should be cautious about easy switching to specialized formula without definite diagnosis of CMA.

A major limitation of the present study was that the data were obtained primarily from self-administered questionnaires. First, the details of symptom occurrence and physician's diagnostic process were not collected. The information was lacking about whether the reactions occurred at first or second exposure to CM protein or after some days or weeks of formula use, and how a community physician discriminated between exposure information and outcome assessment, both of which directly related to CM protein. Moreover, the results of serum-specific IgE, skin-prick tests and oral food challenge were not collected. However, at least one of these tests are almost always conducted by physicians who made a diagnosis of CMA at clinics or hospitals in Japan. Second, the exact details of day-by-day feeding status could not be determined from the questionnaires. When mothers sometimes used formula during a period, it would be defined as no use. We could not analyze the influence of such occasional use of formula, which might modify sensitization status to CM protein. Furthermore, if an exclusively breastfed child received formula milk on a certain day during a month and then developed CMA, the infant's mother would be unlikely to administer formula to the infant for some time. In such situations, the mother would answer “the child was breastfed” for that month. Thus, a lack of consumption of formula milk would, at least partially, represent a surrogate for CMA. As shown in Table 2c, however, the significant association between period-2 of formula consumption and CMA at 12 months persisted after the exclusion of children who already had CMA by 6 months. This suggests an influential effect of formula milk although the association could still be explained by reverse causation. Finally, 8130 children were excluded from the analysis

because of a delayed response or missing information. Although the characteristics of these children were virtually similar to those of the children included in the analysis, the differences in symptom occurrence after CM products and introduction of formula and dairy may potentially have led to selection bias.

In conclusion, the present study demonstrates for the first time that regular exposure to formula milk at the age of 3 months or older may have an association with lower CMA at 12 months old in the general Japanese population. Currently, however, the results of this observational study should not be used for promoting formula feeding and never discourage breastfeeding, which has significant benefits for the immune system and neurodevelopment. Ongoing investigations in the JECS cohort, which will continue until the participants reach adolescence, would reveal later outcomes. This study provides a platform to determine the timing and amount of CM protein to be used in future randomized controlled trials aimed at preventing CMA.

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CONFLICTS OF INTEREST

M. Sanefuji and SO report the receipt of a grant and director's fees, respectively, from Morinaga Houshikai, independent of this work. The other authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data set used in this study is available only to researchers who are approved by the Ministry of the Environment, Japan.

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SUPPORTING INFORMATION

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

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Figure S1. Patterns of formula feeding and prevalence of cow's milk allergy using 'exact period'

Period-1 (0–3m)	Period-2 (3–6m)	Period-3 (6–12m)	Pattern	Number		6m-CMA, No. (%)		12m-CMA, No. (%)	
96.0%	65.3%	34.9%	1	20,213	21,074	0 (0)	0 (0)	0 (0)	2 (0.01)
98.6%	86.7%		2	861		0 (0)		2 (0.23)	
100%		88.1%	3	180	2,776	0 (0)	5 (0.18)	0 (0)	56 (2.02)
100%			4	2,596		5 (0.19)		56 (2.16)	
	85.9%	40.6%	5	1,056	1,133	0 (0)	0 (0)	0 (0)	1 (0.09)
	94.5%		6	77		0 (0)		1 (1.30)	
		88.9%	7	931	23,365	9 (0.97)	99 (0.42)	0 (0)	510 (2.18)
			8	22,434		90 (0.40)		510 (2.27)	
			total	48,348		104 (0.22)		569 (1.18)	

Gray and white boxes denote consumption and non-consumption, respectively, of formula milk during each period. The number in the gray boxes represents the percentage of concurrent use of breastmilk in formula-fed children. For each pattern, the prevalence of children with cow's milk allergy (CMA) at 6 and 12 months old is provided. The prevalence in grayed areas is zero as expected from the definition of CMA, which required no consumption of CM products including formula at the evaluation time.

Table S1. Additional analyses on CMA in association with the periods of formula consumption

a. Analysis of 'exact period'

	6 m-CMA (n = 50,332)		12 m-CMA (n = 43,746)	
Formula consumption	aRR (95% CI)[b]	*p*-value	aRR (95% CI)[b]	*p*-value
Period-1 (0–3m)	0.48 (0.18–1.04)	.07	1.05 (0.78–1.38)	.75
Period-2 (3–6 m)	**0.01 (0.00–0.10)[a]**	**< 0.001**	**0.18 (0.05–0.46)**	**< 0.001**
Period-3 (6–12 m)	–	–	**0.01 (0.00–0.05)[a]**	**< 0.001**

b. Inclusion of children who used specialized formula for CMA

	6 m-CMA (n = 74,812)		12 m-CMA (n = 74,812)	
Formula consumption	aRR (95% CI)	*p*-value	aRR (95% CI)	*p*-value
Period-1 (0–3m)	0.92 (0.63–1.31)	.65	1.02 (0.86–1.21)	.79
Period-2 (3–6 m)	**0.02 (0.00–0.05)[a]**	**< .001**	**0.28 (0.17–0.43)**	**< .001**
Period-3 (6–12 m)	–	–	**0.01 (0.00–0.02)[a]**	**< .001**

Note: All the periods of formula consumption and the following covariates were included in the model: sex, maternal allergic diseases, smoking and education, family income, early eczema, and older siblings. Bold text represents statistical significance ($P < 0.05$).

Abbreviations: aRR, adjusted relative risk; CI, confidence interval; CMA, cow's milk allergy

[a]The results was very low as expected from the definition of CMA.

[b]Using Firth's bias reduction method.

Appendix

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