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三株乳酸菌混合物對過敏性皮膚炎的預防效果

Effect of probiotic mix (*Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus*) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial

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Controversy exists regarding the preventive effect of probiotics on the development of eczema or atopic dermatitis. We investigated whether supplementation of probiotics prevents the development of eczema in infants at high risk. In a randomized, double-blind, placebo-controlled trial, 112 pregnant women with a family history of allergic diseases received a once-daily supplement, either a mixture of *Bifidobacterium bifidum* BGN4, *B. lactis* AD011, and *Lactobacillus acidophilus* AD031, or placebo, starting at 4–8 wks before delivery and continuing until 6 months after delivery. Infants were exclusively breast-fed during the first 3 months, and were subsequently fed with breastmilk or cow's milk formula from 4 to 6 months of age. Clinical symptoms of the infants were monitored until 1 yr of age, when the total and specific IgE against common food allergens were measured. A total of 68 infants completed the study. The prevalence of eczema at 1 yr in the probiotic group was significantly lower than in the placebo group (18.2% vs. 40.0%, $p = 0.048$). The cumulative incidence of eczema during the first 12 months was reduced significantly in probiotic group (36.4% vs. 62.9%, $p = 0.029$); however, there was no difference in serum total IgE level or the sensitization against food allergens between the two groups. Prenatal and postnatal supplementation with a mixture of *B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031 is an effective approach in preventing the development of eczema in infants at high risk of allergy during the first year of life.

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Key words: probiotics; *Bifidobacterium; Lactobacillus;* eczema; prevention; infant

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Eczema or atopic dermatitis is a common chronic inflammatory skin disease, mostly occurring in children (1). A recent study has shown that the worldwide prevalence of eczema in childhood was increasing (2), and the prevalence of eczema in Korean children aged 6–12 yrs increased between 1995 (19.7%) and 2000 (27.5%) (3). It is speculated that the increasing prevalence of allergic diseases in developed countries is associated with the so-called 'hygiene hypothesis', in which a lack of infections or other microbial exposures at an early age leads to Th2-dominant immune status and the subsequent development of allergic diseases (4, 5).

Recently, the role of intestinal microflora has been emphasized in the maintenance of normal gut barrier function and development of tolerogenic immune status (6). Mice raised in a germ-free environment failed to develop oral tolerance and had a persistent Th2-dependent immune response, while reconstitution of intestinal microbes during the neonatal period could reverse this immune deviation (7). Infants with allergic diseases showed less intestinal colonization by *Lactobacillus* or *Bifidobacterium* and more colonization by *Clostridium* relative to non-allergic infants (8–10). It is also known that specific intestinal bacteria bring about immune tolerance via the up-regulation of inhibitory Toll-interacting protein (11). These findings suggest that certain gut microbes modulate regulatory T cells, leading to the suppression of allergic disorders (12).

It remains a matter of controversy whether the modification of intestinal microflora by supplementation with probiotic bacteria in early life is effective in preventing eczema. Prenatal and postnatal supplementation with *Lactobacillus rhamnosus* GG has been shown to reduce the prevalence of atopic eczema at 2 yrs of age. This protective effect even continued during 7 yrs of follow-up (13, 14); however, it did not affect the sensitization rate or the development of asthma or allergic rhinitis. In another study, early supplementation with *Lactobacillus acidophilus* (LAVRI-A1) did not reduce the prevalence and severity of atopic dermatitis, but resulted in an increased proportion of infants with allergic sensitization at 1 yr of age (15). Those contradictory findings imply that further studies are needed to assess whether supplementation with probiotic bacteria at an early age has a preventive effect on eczema.

In the present study, we investigated whether prenatal and postnatal administration of a mixture of *Bifidobacteria* and *Lactobacillus* could prevent the development of eczema and sensitization against common food allergens in infants at high risk of atopic disease.

Materials and methods

Study design and participants

This study was a randomized, double-blind, placebo-controlled trial designed to evaluate the preventive effect of probiotics on the development of eczema. A total of 112 pregnant women with a family history of allergic diseases were recruited at Samsung Medical Center (Irwon-dong, Gangnam-gu, Seoul, Korea) from January 2005 through January 2006. A family history of allergic diseases was defined according to the following criteria: (i) when at least one parent or older sibling of the fetus had eczema, as confirmed by a pediatric allergist at enrollment; or (ii) when one of the parents had been diagnosed with asthma and/or allergic rhinitis by a physician, showing house dust mite-specific IgE over 1.0 kU/L by CAP-FEIA immunoassay (Pharmacia, Uppsala, Sweden).

Treatment of either probiotics or placebo was allocated by trials coordinator without detailed knowledge of the clinical history according to computerized randomization. The groups were stratified and block-randomized in accordance with (1) maternal allergy (allergy vs. no allergy), (2) older sibling's eczema, and (3) number of parents affected by allergic disease (1 vs. 2) (Table 1).

Mothers in the probiotics group took a mixture of *Bifidobacterium bifidum* BGN4 [1.6×10^9 colony forming units (CFU)], *Bifidobacterium lactis* AD011 (1.6×10^9 CFU), and *Lactobacillus acidophilus* AD031 (1.6×10^9 CFU) in 0.72 g of maltodextrin and 0.8 g of alpha-corn (Bifido Inc., Hongchungun, Korea) once daily from 8 wks before the expected delivery to 3 months after delivery. Infants were fed the same powder dissolved in breast milk, infant formula, or sterile water from 4 to 6 months of age. Mothers and infants in the placebo group took maltodextrin and alpha-corn without probiotic bacteria. The

Table 1. Baseline characteristics of the participants at the time of randomization

	Probiotics group n (%)	Placebo group n (%)
Enrolled number	57	55
Mother's age (yrs)*	29.93 ± 0.37	29.53 ± 0.45
Maternal allergic diseases	43 (75.4)	45 (81.8)
Parental history of allergic disease		
Biparents	22 (38.6)	14 (25.5)
Single parent	33 (57.9)	38 (69.1)
Allergic diseases in participant's family		
Eczema	34 (59.6)	38 (69.1)
AR or asthma	39 (68.4)	27 (49.1)
House dust mite-specific IgE of parents (kU/L)*		
D. pteronyssinus	6.63 ± 1.37	9.60 ± 2.56
D. farinae	10.41 ± 2.52	12.71 ± 3.01
Paternal smoking	14 (24.6)	19 (34.5)

*Mean ± s.e.m.

There were no significant differences between the groups for any of the variables determined by Student's *t* test for continuous data and Pearson's chi-square test for all nominal data.

AR, allergic rhinitis.

probiotic and placebo sachets and contents looked, smelled, and tasted identical. Compliance was monitored by recording the date at which the administration period was discontinued, and counting the remaining sachets. All mothers were requested to breastfeed their infants for at least 3 months after birth; Thereafter, they were permitted to feed their infants with cow's milk formula. Lactating mothers and infants were prevented from eating peanuts and eggs, as well as yogurt and other probiotic functional foods, during the course of the study.

Subjects were excluded if they met any one of the following exclusion criteria: (1) premature babies delivered at less than 36 wks of gestation; (2) infants with immune deficiency disease, necrotizing enterocolitis, or congenital disorders; or (3) those requiring anticancer treatment or a central venous catheter. In the current study, one infant with a congenital disorder was excluded.

The study protocol was approved by the Ethical Committee at the Samsung Medical Center. Written informed consent was obtained from all participants. Our study protocol was registered in ISRCTN (International Standard Randomised Controlled Trial Number). The registration number is ISRCTN26134979.

Clinical assessments

Infants were followed up to 1 yr of age, involving examinations at 3, 6, and 12 months to assess the occurrence of eczema, the main atopic disease during infancy. Diagnosis of eczema was confirmed when the skin lesions met the criteria of Hanifin and Rajka (16). The severity of eczema was determined using the Six Area Six Sign in Atopic dermatitis (SASSAD) score (17). We also collected data on various clinical histories such as gestational age, birth weight, cesarean section delivery, mother's age, duration of breastfeeding, diet, fever (≥38.5°C), respiratory tract infection, hospitalization, acute gastroenteritis, and use of antibiotics. Diagnosis and clinical assessment of eczema was performed by a pediatric allergist who remained unaware of the actual treatment administered during the entire study period.

Venous blood was obtained at 12 months of age to measure total IgE and specific IgE against common food allergens (egg white, cow's milk, wheat, peanut, soybean, and buckwheat) using CAP-FEIA (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Antigen-specific IgE levels greater than 0.35 kU/L were considered positive. 'Probable egg allergy' was defined when the egg white-specific IgE level was 2 kU/L or higher based on the 95% positive predictive value (18). Likewise, infants were diagnosed as having 'probable cow's milk allergy' when they showed cow's milk-specific IgE of 5 kU/L or higher.

Statistical analysis

We anticipated that this high-risk population would have a 60% cumulative incidence of eczema at an early age, and that intervention with probiotics could reduce the proportion of cases developing allergic diseases to 30%. The study design had more than 80% power at the 5% significance level; the estimated size of each group was 55, which allowed for a 15% dropout rate.

Pearson's chi-square test was used to compare the prevalence of outcome variables and background factors between the two groups. Logistic regression analysis was performed to compare the prevalence of eczema and food allergy, and multivariable logistic regression was used to adjust for potential confounding factors (cesarean delivery, breastfeeding, use of antibiotics). The results are given as Odds Ratio (OR) with a 95% Confidence Interval (CI). Total IgE and allergen-specific IgE of parents were normally distributed and assessed using Student's *t*-test, expressed as mean and standard error of the mean (s.e.m.). All statistical analyses were performed using SPSS 12.0K for Windows (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was considered to be statistically significant.

Results

Characteristics of the participants

A flow chart to show the progress of the present study is displayed in Fig. 1. Among the 159 pregnant women who took a blood test and filled in the questionnaire, 112 who met the inclusion criteria were randomized and divided into the probiotics (n = 57) and placebo (n = 55) groups. Prenatally, no mother discontinued administration of the sachets; overall, 90% of the sachets were administered in both groups. During the postnatal period, two infants in the probiotics group and one in the placebo group were excluded because of poor compliance (less than 70%); approximately 85% of the sachets were administered, with no difference in compliance between the groups. The actually treated prenatal periods were 53.62 ± 1.75 days in probiotics group and 52.29 ± 1.33 days in placebo group. The actual postnatal period of treatment was 180.94 ± 5.21 days in probiotics group and

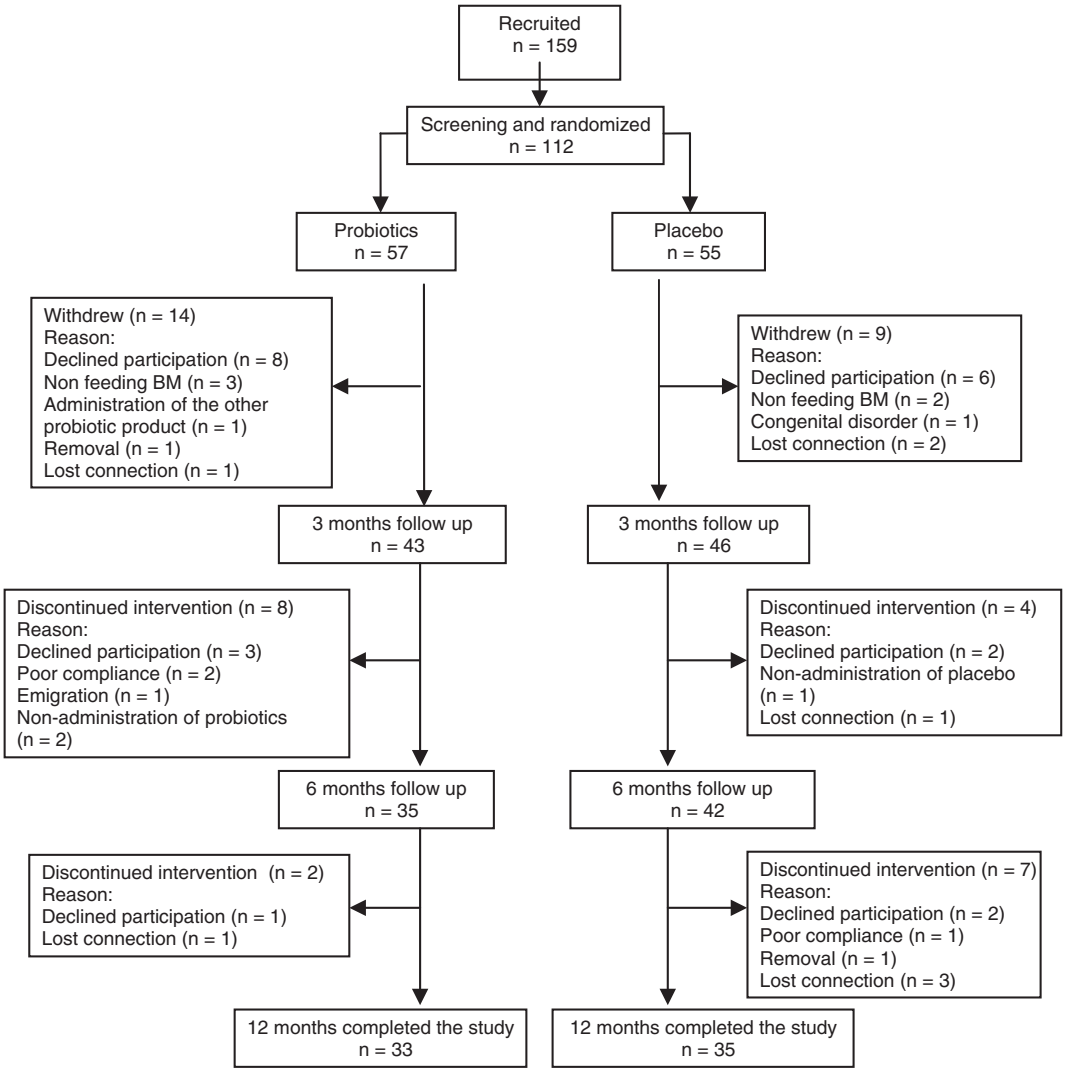

Fig. 1. Flow chart showing the progress of the study.

181.03 ± 5.06 days in placebo group. A total of 44 participants (39.3%) discontinued and ultimately, 68 mothers and their babies (33 in the probiotics group, 35 in the placebo group) completed the study (i.e., participated until the baby was 12 months of age).

There was no difference at the time of randomization between the two groups with respect to mother's age, maternal allergic diseases, biparental allergic diseases, family history of eczema, paternal smoking, or house dust mite-specific IgE levels of parents (Table 1). There was also no significant difference in terms of gestational age, birth weight, gender, presence of older siblings, infections, antibiotic use, and hospitalization during infancy between the two groups when only those participants successfully followed to 12 months were included (Table 2). The rate of cesarean delivery in the probiotics group was half that in the placebo group (15.2% vs. 31.4%; $p = 0.114$), and the total duration of the breastfeeding in the probiotics group was longer than in the placebo group (9.41 ± 0.61 vs. 7.69 ± 0.70 months; $p = 0.068$). Although these findings were not statistically significant, they were considered as potential confounding factors in subsequent analyses.

In the current study, the parents were asked to report any adverse effects whenever they happen. No serious adverse effects developed and although non-specific mild symptoms developed, these were unlikely to have been related to the administration of probiotics.

Effects of probiotics on development of eczema

The prevalence of eczema was not significantly different between the two groups at 3 months of

Table 2. Base and clinical characterization of infants who completed the 1 yr-study

	Probiotics group n (%)	Placebo group n (%)
Number at the end of study	33	35
Gestational age (wks)*	39.66 ± 0.25	39.47 ± 0.19
Birth weight (kg)*	3.33 ± 0.07	3.25 ± 0.06
Gender (boys/girls)	15/18	15/20
Cesarean section delivery	5 (15.2)	11 (31.4)
Duration of breast-feeding (month)*		
Exclusive breast-feeding	5.97 ± 0.89	5.26 ± 0.88
Total breast-feeding	9.41 ± 0.61	7.69 ± 0.70
Presence of older sibling	4 (12.1)	6 (17.1)
Infections during follow-up		
Fever (≥38.5°C)	13 (39.4)	11 (31.4)
Respiratory tract infection	26 (78.8)	26 (74.3)
Acute gastroenteritis	11 (33.3)	7 (20.0)
Antibiotics use during follow-up	13 (39.4)	11 (31.4)
Hospitalization during follow-up	6 (18.2)	6 (17.1)

*Mean ± s.e.m.
There were no significant differences between the groups for any of the variables determined by Student's *t* test for continuous data and Pearson's chi-square test for all nominal data.

age, up to which point lactating mothers were given the probiotics or placebo (probiotics group, 18.6% vs. placebo group, 34.8%; p = 0.086). At 6 months of age, when the infants were receiving formula supplemented with probiotics or placebo, the prevalence rate of eczema in the probiotics group was half that in the placebo group (20.0% vs. 40.5%; p = 0.053). At 12 months of age, the prevalence rate of eczema in the probiotics group was reduced to less than half that in the placebo group; this result was statistically significant (18.2% vs. 40.0%; p = 0.048; Table 3).The cumulative incidence of eczema in the probiotics group was significantly lower than that in the placebo group at 12 months of age (36.4% vs. 62.9%; p = 0.029; Table 3). This result demonstrated that probiotics protected the infants from developing eczema.

The severity of skin lesions was assessed only in those with eczema by SASSAD, and compared between the two groups. Those without eczema were not scored. There was no significant difference in the severity of eczema between the two groups.

IgE sensitization

Sera were obtained from 31 infants in the probiotics group and 29 in the placebo group to measure total and specific IgE; eight mothers (two from the probiotics group, six from the placebo group) refused to withdraw blood from their infants. Total IgE level and the frequency of sensitization against common food allergens was similar in both groups, suggesting that probiotics did not affect sensitization (Table 4). The prevalence rate of IgE-associated eczema or atopic eczema in the probiotics group was half that in the placebo group, although this was not significantly different (9.7% vs. 20.7%, p = 0.233). 'Probable egg allergy' or 'probable cow's milk allergy' occurred similarly in both groups (Table 4).

Discussion

In this double-blind, randomized, placebo-controlled study, we demonstrated the preventive effect of mixed probiotics (*B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031) on development of eczema in infants at high risk of atopic diseases. *B. bifidum, B. lactis*, and *L. acidophilus* have been detected in samples from healthy humans (19, 20), and these probiotic bacteria were used in our study. *B. bifidum* BGN4 exhibited a prominent adhesive capacity for intestinal epithelial cells, which is one of the desirable properties for a probiotic effect (21). In the CD4 + $CD45RB^{high}$ T cell transfer inflammatory bowel disease model, *B. bifidum*

Table 3. Cross-sectional prevalence and cumulative incidence of eczema at 3, 6, and 12 months of age

	Probiotics group	Placebo group	p-value	Adjusted OR(95% CI)	p-value
Cross-sectional prevalence					
3 months	8/43 (18.6%)	16/46 (34.8%)	0.086	0.511 (0.178–1.468)	0.212
6 months	7/35 (20.0%)	17/42 (40.5%)	0.053	0.377(0.119–1.197)	0.098
12 months	6/33 (18.2%)	14/35 (40.0%)	0.048*	0.256(0.067–0.976)	0.046†
Cumulative incidence at 12 months	12/33 (36.4%)	22/35 (62.9%)	0.029*	0.243(0.075–0.792)	0.019†

*Significant difference between the groups as determined by Pearson's chi-square test.
†p value was calculated by multivariable logistic regression analysis adjusted for the antibiotics use, total duration of breastfeeding, and delivery by cesarean section.

Table 4. Comparison of sensitization and prevalence of probable food allergy between two groups

	Probiotics group n (%)	Placebo group n (%)	p-value
Number who completed blood test	31	29	
Total Serum IgE (kU/L)*	111.7 ± 58.9	80.6 ± 28.7	0.638
Food-specific IgE (≥0.35 kU/L)			
Any food	12 (38.7)	15 (51.7)	0.311
Egg white	9 (29.0)	8 (27.6)	0.901
Cow's milk	9 (29.0)	11 (37.9)	0.465
Soybean	3 (9.7)	3 (10.3)	0.931
Wheat	2 (6.5)	2 (6.9)	0.946
Peanut	1 (3.2)	4 (13.8)	0.139
Buckwheat	1 (3.2)	2 (6.9)	0.514
IgE-associated eczema	3 (9.7)	6 (20.7)	0.233
Probable food allergy			
Egg	4 (12.9)	4 (13.8)	0.919
Cow's milk	1 (3.2)	0 (0.0)	0.329

*Mean ± s.e.m.
There were no significant differences between the groups for any of the variables determined by Student's *t* test for continuous data and Pearson's chi-square test for all nominal data.

BGN4-fed mice showed suppression of inflammatory cell infiltration and reduced levels of CD4 + T lymphocyte infiltration and inflammatory cytokine production compared with skim milk-fed mice (22). Orally administered *B. bifidum* BGN4 also prevented IgE-mediated ovalbumin-induced allergy in C3H/HeJ mice (23). These findings suggest that *B. bifidum* BGN4 supplementation could be helpful in the control of aberrant immune responses.

In our preliminary study, mice fed with *B. lactis* AD011 and *L. acidophilus* AD031 showed suppressed levels of ovalbumin-specific IgE in serum, reduced concentrations of IL-4 and increased concentrations of IL-10 and IFN-γ in spleen cell culture assay on ovalbumin-induced allergy model (24). Additionally, *B. lactis* AD011 and *L. acidophilus* AD031 increased the production of IL-10 in dendritic cells (not published). Previous studies also have reported that the administration of *B. lactis* or *L. acidophilus* species alleviated the symptoms of allergic diseases. For example, supplementation with *B. lactis* Bb-12 reduced the severity of atopic eczema in infants (25), and oral administration of fermented milk containing *L. acidophilus* L-92 improved allergic rhinitis (26). Based on these results, we attempted to prevent the development of eczema with mixture of beneficial probiotics (*B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031), and found these probiotics could be used for the infants at high risk of developing eczema.

Recent study revealed that supplementation with *L. acidophilus* (LAVRI-A1) did not prevent atopic dermatitis, but instead led to an increased frequency of common antigen sensitization in infants at high risk of allergy by 1 yr of age (15). This contradictory result may be attributed to different study population, the use of different strain or no prenatal administration of probiotics, despite using the same species, *L. acidophilus*. However, further studies are necessary to verify which species or strains are most beneficial, because our study used mixture of those probiotics, not a single strain.

Several clinical trials have investigated whether prenatal or indirect supplementation with probiotics via breastfeeding could enhance the primary prevention of eczema at an early age. When prenatal and breastfeeding mothers were supplemented with *L. rhamnosus* GG from 2–4 wks before delivery, the rate of their infants' atopic eczema at 2 yrs of age was half that of those supplemented with the placebo (13). Infants born from mothers supplemented with *L. reuteri* for 4 wks before delivery showed a lower frequency of IgE-associated eczema and positive reaction to a skin prick test than the placebo group (27). The above results may well suggest that maternal immunocompetence has an effect *in utero* or on breastfeeding infants. Recent study showed that maternal probiotic supplementation reduced sensitization in infants at high risk of developing allergic diseases and that might have been related to the change in the composition of breast milk such as TGF-β2 (28).

In this prospective study, the frequency of positive food antigen-specific IgE sensitization and food allergy in Korean infants at high risk was not reduced by supplementation with probiotics. This result was consistent with that of the previous study (29), although the reason for the discrepancy between development of eczema and sensitization against food allergens was not clear. However, our data showed that sensitization against any one of common food allergen appeared to be lower in probiotics group (38.7%) than in placebo group (51.7%). The prevalence of IgE-associated eczema also seemed lower in probiotics group (9.7% vs. 20.7%). Still it might be possible to demonstrate the effect of probiotics on the prevention of sensitization if investigations with larger study population are conducted.

Our study is limited by the high drop-out rate. Some participants stopped participating in the study without explanation, or moved from the area. In addition, we excluded all participants who did not adhere to our protocol. Consequently, a high proportion of participants failed

to complete the study. Intention-to-treat analysis may be helpful in such a situation; however, the full application of intention-to-treat analysis is possible only when complete outcome data are available for all randomized subjects. In the present study, however, data are missing regarding the primary outcome for discontinued participants, and the methods employed to deal with this problem were inadequate, potentially leading to bias. We analyzed a total of 44 participants who discontinued or withdrew from the study. Twenty-three participants dropped out before the first follow-up, and their development of eczema was not identified. Among 21 infants who discontinued the study after 3 months of age, the occurrence of eczema was 20.0% (2/10) in the probiotics group and 54.5% (6/11) in the placebo group at their last visit. Considering that the prevalence or cumulative incidence of eczema in the probiotics group is less than half that in the placebo group, the administration of probiotics in the discontinued participants appears to have had a similar preventive effect to that in those who completed the study. In addition, among those who successfully completed the 1-yr study, there was no difference in baseline characteristics or drop-out rate between the probiotics group and placebo group. Therefore, our results suggest that a probiotic mixture was beneficial in preventing eczema, despite the high drop-out rate.

This study showed high prevalence of eczema. The prevalence (40.0%) at 1 yr and cumulative incidence (62.9%) of eczema in placebo group seemed high in the present study. Other studies also showed that the frequency of eczema in placebo group was as high as 45–46% (10, 13). We obtained similar data in our previous study where the prevalence of eczema was 59% and 41% in cow's milk formula-fed group and breastmilk-fed group, respectively, in infants at high risk of eczema (30). Probably high incidence of eczema might have been due to the selection of infants with high risk of developing atopic disease.

Additionally, our study ended when the infants were 1 yr of age. Although we could find lower incidence of eczema in probiotics group, it is not clear whether this preventive effect persists as they grow older.

In conclusion, mixture of probiotics (*B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031) have beneficial effect to prevent development of eczema in infants at high risk during their first year of life. Further studies are needed to understand the basic mechanisms of probiotics in the primary prevention of eczema.

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