

Letter to the Editor

Early probiotic supplementation for allergy prevention: Long-term outcomes

To the Editor:

Progressive change in the patterns and diversity of microbial exposure in early life remains a leading candidate in the rise in many inflammatory diseases. Initial attempts to redress this consequence of modern lifestyle change by using probiotic strains have met with some degree of success in preventing common inflammatory conditions such as allergic diseases. However, much research is still needed to more clearly define optimal early colonization profiles and the best strategies to achieve these. A critical part of this process is understanding long-term and strain-dependent effects, and it is therefore essential that long-term outcomes of early interventions are reported. So far, only 2 randomized placebo-controlled studies have examined long-term (ie, ≥ 5 years) outcomes.¹⁻⁴ Both studies used a high-risk cohort and supplemented the children with probiotics pre- (4 weeks) and postnatally (6 months); the study by Kalliomaki et al¹⁻³ used *Lactobacillus rhamnosus* GG, which significantly reduced the risk of developing eczema during the first 7 years of life; the study by Kuitunen et al⁴ used a probiotic mixture (2 lactobacilli, 1 *Bifidobacterium*, and 1 *Propionibacterium*), which significantly reduced the risk of developing IgE-associated allergic diseases, namely, sensitization and eczema, but only for cesarean-delivered children. Here, we report the long-term effects of postnatal supplementation with *Lactobacillus acidophilus* (LAFTI L10/LAVRI-A1), which was paradoxically associated with an increased risk of sensitization in the first year of life.^{5,6} As noted in the original study, the high-risk children in this cohort were randomized to receive *L. acidophilus* or maltodextrin placebo from birth for the first 6 months of life.⁵ At 5 years of age, all initial participants were invited to a follow-up visit to assess doctor-diagnosed eczema, IgE-mediated food allergy, allergic rhinitis, asthma, and sensitization to common food and aeroallergens, as described more fully in this article's Online Repository at www.jacionline.org.

Of the 178 children who completed the supplementation period, 123 attended this 5-year visit, corresponding to a follow-up rate of 69%. Although the loss to follow-up is typical of that reported in other recent long-term randomized control studies,^{E5} the reduction in power (to 70%; see the Online Repository) requires conservative interpretation of the findings. The number and proportion of children who had received the probiotic (n = 66; 54%) and the placebo (n = 57; 46%) were comparable (see Fig E1 in this article's Online Repository at www.jacionline.org), and there were no major differences in the baseline characteristics of the children who were lost to follow-up (n = 55) compared with completers (n = 178) (see Table E1 in this article's Online Repository at www.jacionline.org).

At 5 years of age, there were no significant differences ($P = .46$) in the proportion of children with a current diagnosis of any doctor-diagnosed allergic diseases in the probiotic (n = 28 of 63; 44%) compared with the control group (21 of 56; 38%), although there was a subtle trend for more children in the probiotic group with doctor-diagnosed eczema ($P = .08$) and recurrent wheezing ($P = .06$) (Table I). There were no significant differences in sensitization (Table I). This is consistent with previous

TABLE I. Clinical characteristics of children at 5 years of age: probiotic compared with placebo group

Characteristic*	Control (n = 57)	Probiotic (n = 66)	P value
Diagnosed allergic disease (any)	21/56 (38)	28/63 (44)	.46
Sensitization (SPT+ to any allergens)	20/45 (44)	30/57 (53)	.43
Food (any)	6/45 (13)	13/57 (23)	.31
Egg (whole fresh egg)	3/45 (7)	6/57 (11)	.73
Cow's milk	4/45 (9)	3/57 (5)	.70
Peanut	4/45 (9)	11/57 (19)	.17
Inhalant (any)	21/45 (47)	30/57 (53)	.70
House-dust mite	13/45 (29)	20/57 (35)	.53
Grass mix	11/45 (24)	13/57 (23)	1.00
Mould mix	8/45 (18)	11/57 (19)	1.00
Cat pelt	5/45 (11)	12/57 (21)	.29
Rye grass	12/45 (27)	16/57 (28)	1.00
Eczema			
Any doctor-diagnosed eczema	9/56 (16)†	19/62 (31)†	.08†
SPT+ and eczema	7/54 (13)	13/60 (22)	.32
SCORAD at 5 y, mean $\pm$ SEM	13.35 $\pm$ 2.80	13.31 $\pm$ 3.45	.99
SCORAD at worst in last year, mean $\pm$ SEM	26.41 $\pm$ 2.19	30.69 $\pm$ 3.37	.33
Respiratory allergy			
Allergic rhinitis and inhalant SPT+	9/50 (18)	8/58 (14)	.60
Diagnosed asthma	5/57 (9)	8/66 (12)	.77
Recurrent cough (without cold)	15/57 (26)	26/65 (40)	.13
Wheeze the last year	6/53 (11)	14/64 (22)	.15
Wheeze ever	12/57 (21)	17/66 (26)	.67
Recurrent wheeze ≥ 2 episodes	0/38 (0)†	5/48 (10)†	.06†
Food allergy (IgE mediated)	3/51 (6)	8/64 (13)	.34

Unless otherwise stated, values are presented as number of children/sample size (%). SCORAD, Scoring Atopic Dermatitis; SPT, skin prick test.

*All outcomes refer to "recent" symptoms (in the preceding 12 mo) unless otherwise stated.

†Indicates relationships of interest (although not statistically significant).

reports on this cohort^{5,6} that this probiotic failed to show any clinical benefits on allergy outcomes at earlier ages, and the longitudinal data are summarized in Table E2 in this article's Online Repository at www.jacionline.org (unadjusted) and Table II (adjusted for the potential confounding influences identified in Table E3 in this article's Online Repository at www.jacionline.org, which did not alter the findings).

While inevitable dropout rates place some limitation on the interpretation of long-term follow-up studies, these findings nonetheless provide information on long-term effects of early probiotic supplementation for allergy prevention. Consistent with the previous reports from this study, regular dosing with this *L. acidophilus* strain from 0 to 6 months of age had no long-term benefits. The significantly higher rates of sensitization seen at 1 year of age in the probiotic group⁵ were no longer apparent in the subsequent cumulative analysis (Table II). In the 2 other long-term studies by Kalliomaki et al¹⁻³ and Kuitunen et al,⁴ the only beneficial effects found (a decrease in eczema in children supplemented with *L. rhamnosus* GG and a decrease in IgE-associated allergic diseases in cesarean-delivered children supplemented with a probiotic mixture, respectively) remained evident in the follow-up analysis. In both of these studies, the supplements were administered in both the prenatal and the postnatal

TABLE II. Adjusted* odds ratios (ORs) and adjusted* hazard risk (HR) with 95% CIs for the main clinical outcomes in children attending year-1, year-2.5, and year-5 visits

Clinical outcome	Year 1 (n = 175)†			Year 2.5 (n = 153)‡			Year 5 (n = 123)‡			Cumulative, years 1-5		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	HR	95% CI	P value
Diagnosed allergic disease (any)	1.26	0.67-2.37	.47	1.34	0.68-2.63	.40	0.956	0.44-2.08	.91	1.07	0.80-1.44	.63
Doctor-diagnosed eczema (any)	1.11	0.59-2.11	.74	1.49	0.74-2.99	.26	1.95	0.76-5.03	.17	1.17	0.83-1.63	.37
Sensitized (SPT+)	2.0§	1.05-4.09§	.04§	1.32	0.62-2.78	.47	1.27	0.55-2.93	.58	1.27	0.91-1.78	.17
SPT+ and eczema	2.0§	0.89-4.48§	.10§	1.48	0.63-3.50	.37	1.70	0.59-4.92	.33	1.5§	0.96-2.42§	.07§
Food allergy (IgE mediated)	1.65	0.65-4.22	.30	0.85	0.33-2.20	.73	1.83	0.43-7.78	.41	1.17	0.67-2.02	.58
Diagnosed asthma	—	—	—	1.55	0.38-6.23	.54	1.40	0.39-5.00	.61	—	—	—
Allergic rhinitis and inhalant SPT+	—	—	—	—	—	—	0.506	0.16-1.61	.25	—	—	—

Boldface indicates statistically significant relationship.

SPT, Skin prick test.

*Adjusted for delivery mode, birth length, head circumference at birth, and maternal age.

†Clinical history in the preceding year.

‡Outcomes at follow-up visits refer to clinical characteristics since the preceding visit.

§Shaded fields indicate relationships of interest.

period, whereas in our study the probiotic was administered only after birth. This has led to speculation that antenatal supplementation is required for optimal effect. However, studies using only postnatal supplementation demonstrated both immunomodulatory effects and eczema prevention⁷ while a study using prenatal *L. rhamnosus* GG found no effect.⁸ Thus, in addition to strain-specific effects,⁹ there are likely to be different effects of the timing of administration, which require further research. Strategies to restore favorable patterns and diversity of enteric microflora remain an important avenue for allergy prevention.

We acknowledge the staff and volunteers who assisted in this study. We are particularly grateful to the staff in the Children's Allergy Immunology Research (CAIR) team, and the obstetricians and midwives at St John of God Hospital, Subiaco and Murdoch; Mercy Hospital, Mt Lawley; and King Edward Memorial Hospital, Subiaco, Western Australia.

Marie P. Jensen, PhD^{a,b}

Suzanne Meldrum, PhD^a

Angie L. Taylor, PhD^a

Janet A. Dunstan, PhD^a

Susan L. Prescott, PhD, MD^a

From ^athe School of Paediatrics and Child Health, University of Western Australia, Perth, Australia; and ^bthe Faculty of Science, Aarhus University, Aarhus, Denmark. E-mail: sprescott@meddent.uwa.edu.au.

This project was supported by funds from the National Health and Medical Research Council of Australia. M.P.J. was supported by the Villum Kann Rasmussen Foundation, Denmark.

Disclosure of potential conflict of interest: M. P. Jensen has received research support from the Villum Kann Rasmussen Foundation. S. L. Prescott has received research

support from the National Health and Medical Research Council of Australia. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;357:1076-9.
2. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003;361:1869-71.
3. Kalliomaki M, Salminen S, Poussa T, Isolauri E. Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007;119:1019-21.
4. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009;123:335-41.
5. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007;119:184-91.
6. Prescott SL, Wiltchut J, Taylor A, Westcott L, Jung W, Currie H, et al. Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. *Allergy* 2008;63:1481-90.
7. West CE, Hammarström ML, Hernell O. Probiotics during weaning reduce the incidence of eczema. *Pediatr Allergy Immunol* 2009;20:430-7.
8. Boyle RJ, Ismail IH, Kivivuori S, Licciardi PV, Robins-Browne RM, Mah LJ, et al. *Lactobacillus* GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial. *Allergy* 2011;66:509-16.
9. Wickens K, Black PN, Stanley TV, Mitchell E, Fitzharris P, Tannock GW, et al. A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2008;122:788-94.

<http://dx.doi.org/10.1016/j.jaci.2012.07.018>

METHODS

Study design

The original study has been described in detail previously.^{E1} Briefly, infants with atopic mothers were randomly assigned within 48 hours of birth to receive placebo (maltodextrin dissolved in 1–2 mL of water) or 3×10^9 colony-forming units of *Lactobacillus acidophilus* LAFTI L10 (also known as LAVRI-A1) in maltodextrin dissolved in 1 to 2 mL of water on a daily basis to 6 months of age. A total of 178 infants completed the supplementation. Clinical outcomes have been previously assessed at 1 and 2.5 years,^{E1,E2} and the same methods were used to assess the longer-term allergic outcomes at 5 years of age. Fig E1 shows the number of study participants followed over the course of the study. The study was approved by the ethics committee at the Princess Margaret Hospital as previously indicated.^{E1,E2}

Clinical outcomes

Children were clinically evaluated at 5 years of age by the same pediatrician as at the earlier visits (Prof S. L. Prescott), and the visit included a detailed history, allergy skin prick testing, and physical examinations. A child was classified as having “allergic disease” if he or she had a physician diagnosis of IgE-mediated food allergy, eczema, allergic rhinitis, or asthma at these assessments. The diagnosis of eczema was made in infants with typical skin lesions responsive to topical steroids,^{E3} and severity was determined by using the Scoring Atopic Dermatitis index.^{E4} Asthma was diagnosed if the child had recurrent wheeze (>2 episodes in the preceding 12 months) and responded to bronchodilator therapy. A diagnosis of allergic rhinitis was made if the child had a clinical history suggestive of allergic rhinitis with evidence of sensitization to inhalants. IgE-mediated food allergy was defined as a history of immediate (within 60 minutes) symptoms after contact with and/or ingestion of food and a positive skin prick test result to the implicated food. Skin prick tests with cow’s milk, peanut, house dust mite, cat, grass, mold (Hollister-Stier Laboratories, Spokane, Wash), and whole raw egg were performed. Histamine and glycerine served as positive and negative control, respectively. A wheal diameter of 3 mm or more was considered positive. In addition to the clinical visits, parents recorded data on other symptoms and environmental factors on diary cards.

Assessment of potential confounding factors and interpretation of clinical data

Information on potential confounding factors was collected prospectively (diary cards) and included information on other clinical disease (vaccination, infection, day care, diet, and medication use including antibiotics) and environmental factors (including carpeting, sibship, and pets).

Statistical analyses

Data were analyzed with SPSS software version 19 (SPSS Institute, Inc, Chicago, Ill). Differences between the probiotic group and the placebo group for categorical data were determined by Fisher exact tests or logistic regression. Logistic regression was used to determine the odds ratio of developing each binary allergic outcome in the probiotic group compared with the placebo after adjusting for potential confounding factors (including delivery mode, birth length, birth head circumference, and maternal age). Multivariate Cox proportional hazards regression modeling was used to estimate hazard ratios of developing main allergic outcomes over time and adjusted for the same potential confounding factors included in the logistic regression model. Continuous data were normally distributed and assessed by using Student *t* test, expressed as means and SEM. A *P* value of less than .5 was considered statistically significant. A *post hoc* Wilcoxon-Mann-Whitney power analysis was based on our previous assumption^{E1} where an intervention would reduce the risk of developing allergy by 20% with a relative risk of 0.7 and $\alpha = .05$. The original study, which aimed for

90 subjects in each study group, had more than 80% power to detect this difference in atopic disease.

RESULTS

Population characteristics

As noted in the main article, 123 of the 178 children who completed the original study supplementation period attended this 5-year visit, corresponding to a follow-up rate of 69%, which is typical of that reported in other recent long-term randomized control studies.^{E5} A *post hoc* power analysis of this sample size revealed 70% power to detect a 20% fall in the expression of allergy and that a total sample size of 160 had been required to obtain 80% power. This inevitably limits the interpretation of the data from the 5-year visit, and, furthermore, it is difficult to exclude bias in the follow-up population (potentially with higher retention of children with allergic outcomes), and the results have therefore been interpreted conservatively. Although the outcomes of these individuals are not known, the baseline characteristics of the “dropouts” at both the 2.5-year assessment (*n* = 25, 14%) and the 5-year assessment (*n* = 55, 31%) are compared with the original study population in Table E1, with no significant differences identified.

The number and proportion of 5-year-old children who had received the probiotic and the placebo was *n* = 66 (54%) and *n* = 57 (46%), respectively (Fig E1). In the 5-year-old children, we also compared for differences in baseline characteristics between the probiotic and the control groups to identify potential confounding factors (Table E3). There were no major differences in sex, gestation weeks, birth weight, parity, maternal antibiotic usage during breast-feeding, and paternal atopic status. There were also no differences in environmental factors such as dietary patterns, antibiotic usage, and pets, and childcare attendance did not differ significantly between the 2 groups. However, there were considerable differences in maternal age, birth length, and birth head circumference between the 2 groups and a higher proportion of children on probiotics were delivered vaginally (a recognized protective factor from allergic disease^{E6}). As indicated, we adjusted for any potential confounding effects of these effects in regression analysis, with the adjusted odds ratios and the adjusted hazard ratios for each allergic outcome in relation to the intervention (Table I). Notably, this did not change the findings.

REFERENCES

- E1. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007;119:184–91.
- E2. Prescott SL, Wiltshut J, Taylor A, Westcott L, Jung W, Currie H, et al. Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. *Allergy* 2008;63:1481–90.
- E3. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44–7.
- E4. Anonymous. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993;186:23–31.
- E5. Fewtrell MS, Kennedy K, Singhal A, Martin RM, Ness A, Hadders-Algra M, et al. How much loss to follow-up is acceptable in long-term randomised trials and prospective studies? *Arch Dis Child* 2008;93:458–61.
- E6. Pistiner M, Gold DR, Abdulkarim H, Hoffman E, Celedon JC. Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. *J Allergy Clin Immunol* 2008;122:274–9.

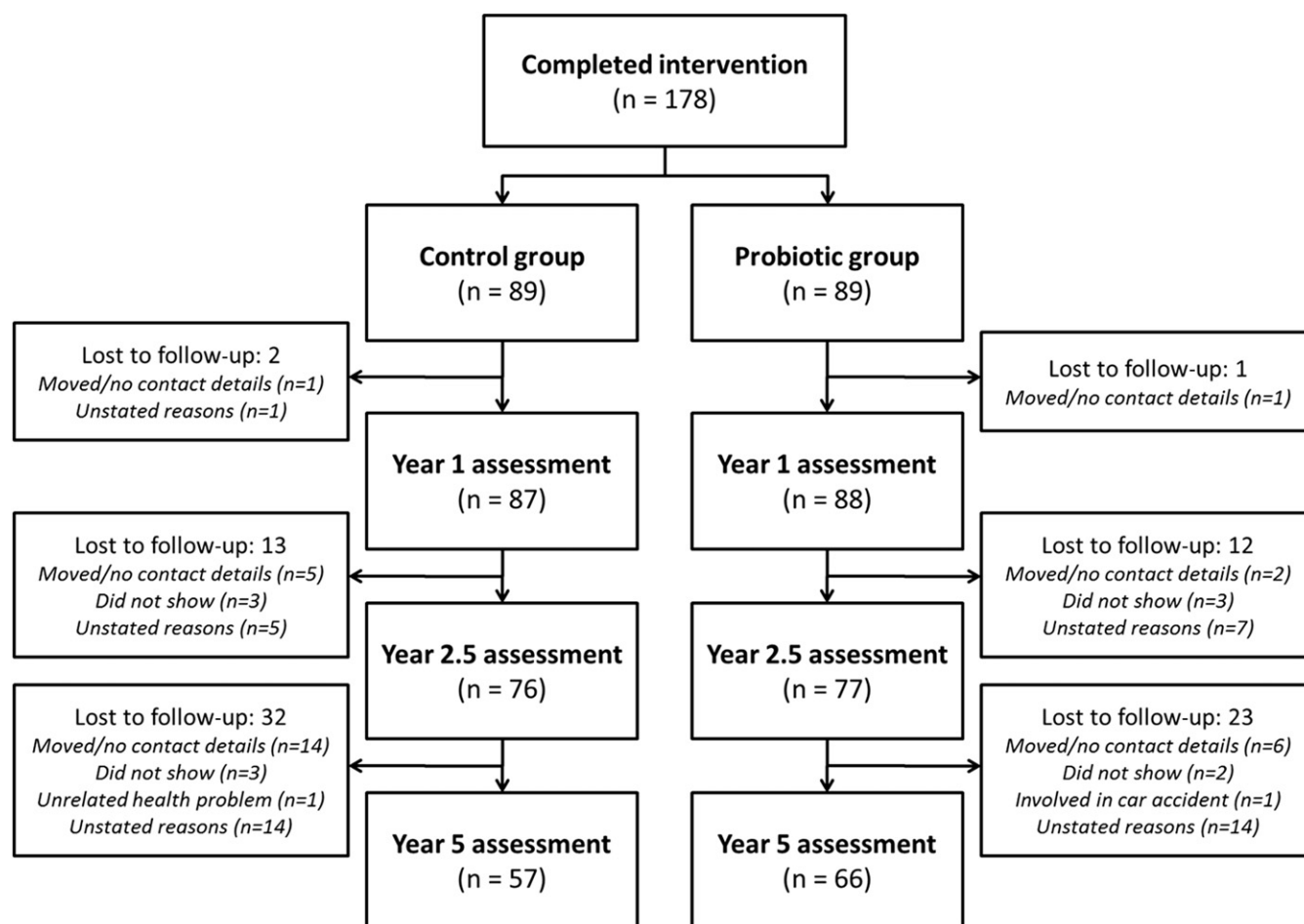


FIG E1. Flowchart showing the number of study participants over time.

TABLE E1. Baseline characteristics of dropouts at 2.5 and 5 years compared with children completing the intervention

Characteristic	Completed intervention (n = 178)	Year 2.5 dropouts (n = 25)		Year 5 dropouts (n = 55)	
	n (%)	n (%)	P value	n (%)	P value
At birth					
Probiotic group	89/178 (50)	11/25 (44)	.67	22/55 (40)	.28
Boys	87/178 (49)	13/25 (52)	.83	30/55 (55)	.54
Gestation weeks, mean $\pm$ SEM	39.38 $\pm$ 0.81	39.34 $\pm$ 0.23	.84	39.37 $\pm$ 0.15	.95
Vaginal delivery	98/178 (55)	13/25 (52)	.83	27/55 (49)	.44
Weight (g), mean $\pm$ SEM	3466.32 $\pm$ 32.54	3632.12 $\pm$ 92.59	.08	3479.85 $\pm$ 56.17	.84
Length (cm), mean $\pm$ SEM	50.04 $\pm$ 0.16	50.14 $\pm$ 0.43	.83	50.05 $\pm$ 0.30	.99
Head circumference (cm), mean $\pm$ SEM	34.99 $\pm$ 0.10	35.40 $\pm$ 0.25	.13	34.97 $\pm$ 0.19	.94
Parity	83/178 (47)	12/25 (48)	1.00	24/55 (44)	.76
Maternal age (y), mean $\pm$ SEM	32.82 $\pm$ 0.37	32.24 $\pm$ 0.86	.58	31.89 $\pm$ 0.71	.23
Paternal atopy	86/176 (49)	9/25 (19)	.29	25/54 (46)	.76
During first 6 mo of life					
Breast-fed ever	175/178 (98)	25/25 (100)	1.00	54/55 (98)	1.00
Ever had formula	123/176 (70)	16/25 (64)	.64	39/54 (72)	.87
Maternal antibiotics while breast-feeding	74/176 (42)	9/25 (36)	.67	22/54 (41)	1.00
Ever had antibiotics	39/173 (23)	7/25 (28)	.61	12/54 (22)	1.00
Pets in household	99/176 (49)	18/25 (72)	.19	28/54 (52)	.64
Play group attendance	113/176 (64)	14/25 (56)	.51	35/54 (65)	1.00
Day care attendance	16/176 (9)	1/23 (4)	.70	2/54 (4)	.26

Unless otherwise stated, values are presented as number of children/sample size (%).

TABLE E2. Unadjusted odds ratios (ORs) and unadjusted hazard ratios (HRs) and 95% CIs for the main clinical outcomes in children attending year-1, year-2.5, and year-5 visit

Clinical outcome	Year 1 (n = 175)*			Year 2.5 (n = 153)†			Year 5 (n = 123)‡			Cumulative, years 1-5		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	HR	95% CI	P value
Diagnosed allergic disease (any)	1.29	0.71-2.34	.40	1.41	0.75-2.68	.29	1.33	0.64-2.78	.44	1.12	0.85-1.47	.43
Doctor-diagnosed eczema (any)	1.19	0.65-2.17	.58	1.56	0.81-3.02	.18	<i>2.31‡</i>	<i>0.94-5.64‡</i>	<i>.07‡</i>	1.23	0.90-1.69	.20
Sensitized (SPT+)	2.04	1.07-3.92	.03	1.25	0.61-2.56	.54	1.39	0.63-3.05	.41	1.267	0.92-1.76	.16
SPT+ and eczema	2.18	1.01-4.73	.045	1.52	0.67-3.45	.32	1.86	0.68-5.07	.23	1.61	1.03-2.51	.04
Food allergy (IgE mediated)	1.64	0.67-4.02	.28	1.00	0.41-2.47	1.00	2.29	0.57-9.10	.24	1.29	0.76-2.19	.35
Diagnosed asthma	—	—	—	1.42	0.43-4.69	.57	1.43	0.44-4.66	.55	—	—	—
Allergic rhinitis and inhalant SPT+	—	—	—	—	—	—	0.73	0.26-2.06	.55	—	—	—

Boldface indicates statistically significant relationship.

SPT, Skin prick test.

*Clinical history in the preceding year.

†Outcomes at follow-up visits refer to clinical characteristics since the preceding visit.

‡Italicized fields indicate relationships of interest.

TABLE E3. Baseline characteristics of children completing year-1, year-2.5, or year-5 clinics

Characteristic	Year 1			Year 2.5			Year 5		
	Control (n = 87)	Probiotic (n = 88)	P value	Control (n = 76)	Probiotic (n = 77)	P value	Control (n = 57)	Probiotic (n = 66)	P value
At birth									
Boys	44/87 (51)	42/88 (48)	.76	39/76 (51)	35/77 (45)	.52	27/57 (47)	30/66 (45)	.86
Gestation wk	39.44 (0.11)	39.31 (0.12)	.43	39.49 (0.12)	39.29 (0.13)	.24	39.43 (0.14)	39.36 (0.13)	.72
Vaginal delivery	43/87 (49)	55/88 (63)	.10	36/76 (47)	48/77 (62)	.14	27/54 (48)	44/66 (67)	.04
Weight (g)	3521.05 (44.35)	3419.11 (48.35)	.12	3482.38 (45.12)	3395.72 (51.55)	.21	3514.79 (54.38)	3412.37 (57.67)	.20
Length (cm)	50.46 (0.22)	49.66 (0.24)	.02	50.38 (0.25)	49.67 (0.25)	.045	50.48 (0.28)	49.65 (0.27)	.03
Head circumference	35.20 (0.12)	34.81 (0.15)	.04	35.13 (0.13)	34.71 (0.16)	.04	35.24 (0.14)	34.78 (0.17)	.04
Parity	39/87 (45)	42/88 (48)	.70	34/76 (45)	37/77 (48)	.75	26/57 (46)	33/66 (50)	.72
Maternal antibiotics while breast-feeding 0-6 mo	36/86 (42)	37/88 (42)	1.00	32/74 (43)	33/77 (43)	1.00	22/56 (39)	30/66 (45)	.58
Maternal age (y)	32.37 (0.49)	33.48 (.50)	.12	32.11 (0.58)	33.71 (0.55)	.046	32.86 (0.58)	33.56 (0.61)	.41
Paternal atopy	42/87 (48)	44/87 (51)	.88	37/75 (49)	40/76 (53)	.75	26/57 (46)	35/65 (54)	.47
At respective age									
Ever breast-fed	85/87 (98)	88/89 (99)	.62	74/76 (97)	76/77 (99)	.62	55/57 (96)	66/66 (100)	.21
Ever had formula	73/87 (84)	72/88 (82)	.84	66/76 (87)	61/75 (81)	.38	48/56 (86)	54/66 (82)	.63
Ever had antibiotics	41/87 (47)	49/88 (56)	.29	59/76 (78)	65/76 (86)	.30	52/57 (91)	63/66 (95)	.47
Pets in household	40/86 (47)	46/88 (52)	.45	32/73 (44)	34/74 (46)	.87	24/55 (44)	33/66 (50)	.46
Play group attendance	52/86 (60)	55/88 (63)	.88	39/75 (51)	45/75 (60)	.41	16/52 (31)	17/60 (28)	.84
Day care attendance	26/86 (30)	26/88 (30)	1.00	38/75 (51)	46/75 (61)	.25	23/53 (43)	28/62 (45)	1.00

Unless otherwise stated values are presented as number of children/sample size (%).

Boldface indicates statistically significant relationship.