Perinatal Probiotic Mixture and Development of Allergic Sensitization up to 13 Years of Age

Peldan, Paivi Susanna

2020-04

Peldan , P S , Kukkonen , A K , Savilahti , E & Kuitunen , M 2020 , ' Perinatal Probiotic Mixture and Development of Allergic Sensitization up to 13 Years of Age ' , International Archives of Allergy and Immunology , vol. 181 , no. 4 , pp. 270-277 . https://doi.org/10.1159/000504915

http://hdl.handle.net/10138/350195
https://doi.org/10.1159/000504915

International Archives of Allergy and Immunology

Manuscript:	IAA-2018-11-8/R1 RESUBMISSION
Title:	Perinatal probiotic mixture and development of allergic sensitization up to 13 years of age
Authors(s):	Päivi Peldan (Corresponding Author), Anna Kaarina Kukkonen (Coauthor), Erkki Savilahti (Co-author), Mikael Kuitunen (Co-author)
Keywords:	Allergy prevention, Clinical trial, IgE antibodies specific, Probiotics, Sensitization
Type:	Clinical Allergy – Research Article

Perinatal probiotic mixture and development of allergic sensitization up to 13 years of age

1. Title (and Running head):

Perinatal probiotic mixture and development of allergic sensitization up to 13 years of age (Probiotic and allergic sensitization until 13 years)

2. Authors:

Päivi Peldan[1], Anna Kaarina Kukkonen[2], Erkki Savilahti[1], Mikael Kuitunen[1]

[1] Children´s Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[2] Skin and Allergy Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

3. Corresponding author:

Päivi Susanna Peldan
Children´s Hospital
Pediatric Research Center
University of Helsinki and Helsinki University Hospital
Helsinki, Finland

Address: New Children´s Hospital, Stenbäckinkatu 9, Helsinki, PL 281, 00029 HUS, FINLAND
Telephone number: +358407548667
Email address: paivi.peldan@hus.fi

4. Key words: allergy prevention, clinical trial, IgE antibody-specific, probiotics, sensitization

1. Abstract:

Background

Probiotics have shown promising results in primary prevention of allergies in early years, but long-term effects on allergic sensitization need more evaluation.

Objectives

We conducted a randomized double-blind placebo-controlled study to determine whether the use of a mixture of pre- and probiotics perinatally affects the prevalence of IgE sensitization up to 13 years in highrisk children.

Methods

1223 pregnant women were randomized to receive probiotics or placebo from 36 gestational weeks until delivery, and their infants received pre- and probiotics or placebo from birth until six months. At two, five and 13 years, blood samples were taken to determine specific IgE levels against common foods, pollen and animal antigens.

Results

The prevalence of IgE sensitization to any allergen was high and increased with age. No significant difference in the prevalence of IgE sensitization to any particular one of the tested allergens was found between the groups. At two, five and 13 years these prevalence rates of IgE sensitization to any allergen were 31.1% and 34.1%, 50.1% and 45.6%, and 61.4% and 56.8% in the probiotic and placebo groups, respectively. At 13 years, IgE sensitization to cat/dog dander was more frequent in the probiotic group compared with the placebo group (40.2% vs. 31.0%, p=0.03).

Conclusions

In high-risk children, perinatal use of a mixture of probiotics did not affect the prevalence of sensitization to any one of the tested allergens, but it was associated with more frequent IgE sensitization to cat/dog dander at 13 years.

2. Introduction:

Perinatal use of probiotics may prevent allergic diseases, mainly eczema, in early childhood in high-risk populations (1-6), but results are conflicting, as some studies show no effects (7, 8). Some studies have shown that prenatally administered probiotics can diminish the rate of sensitization (5, 6, 9). Maternal probiotic supplementation (*Lactobacillus rhamnosus [L. rhamnosus]* and *Bifidobacterium longum [B. longum]* or *L. paracasei* and *B. longum*) during pregnancy and breastfeeding had no effect on IgE sensitization in high-risk children at age two (4). Furthermore, at age seven, no difference in sensitization between groups using a perinatal *Lactobacillus* GG (LGG) probiotic strain or placebo was found, although the cumulative risk of developing eczema was diminished (1). We have earlier shown that the use of a mixture of pre- and probiotics perinatally can diminish the prevalence of IgE-associated eczema at age two, and at age five we showed less IgE-associated eczema only in a subgroup of cesarean-delivered children (2, 3). We have previously reported that perinatal use of a mixture of probiotics decreased the prevalence of eczema up to 10 years of age but the prevalence of allergic rhinitis was increased at 5–10 years (10). In another study, in high-risk children, perinatal use of *L. reuteri* prevented sensitization and eczema at age two but it had no effect on atopic sensitization or allergic disease at age seven (5). Perinatal use of *L. rhamnosus* has been associated with lower prevalence rates of sensitization and eczema in high-risk children from birth to age six (6).

In this randomized double-blind placebo-controlled trial we evaluated the longitudinal effect of perinatally administered pro- and prebiotics on the prevalence of IgE sensitization at two, five and 13 years.

3. Materials and Methods:

Study design and participants

The design of the study has been detailed previously (2,3). Children in the randomized, double-blind and placebo-controlled study were born between November 2000 and March 2003. The study population was collected in the Helsinki area. 1223 pregnant women carrying a fetus with a high-risk of allergy (at least one of the parents had physician-diagnosed allergic disease) were randomized to receive probiotics or placebo at 35 weeks. A mixture of probiotics (LGG, ATCC 53103, 5×10^9 colony-forming units [cfu], *L. rhamnosus* LC705, DSM 7061, 5×10^9 cfu, *Bifidobacterium breve* Bb99, DSM 13692, 2×10^8 cfu, and *Propionibacterium freudenreichii ssp. shermanii* JS, DSM 7076, 2×10^9 cfu) or placebo was taken by mothers from 36 weeks twice daily until delivery. The same capsules were opened and mixed with syrup containing prebiotics (0.8 g of galacto-oligosaccharides) and given to the infants once daily from birth to six months. In the placebo group capsules without pre- and probiotics looked and smelled the same as the test capsules. Children with major malformations, children born at less than 37 weeks of gestation and b-twins were excluded. Follow up lasted 13 years.

Specific IgE analysis

Blood samples were drawn from the umbilical cord after delivery, and at two, five and 13 years to determine specific IgE levels. At all time-points levels of specific IgEs to food (cow´s milk and egg), pollen (birch and timothy grass) and animals (cat and dog) were analyzed using the ImmunoCAP system (Thermo Fisher, Uppsala, Sweden). We used the term “any specific IgE ≥0.35 kU/l” to indicate that the level of any particular one of the specific IgEs in the blood samples was ≥0.35 kU/l. In addition, at five years, levels of IgEs to peanuts and house-dust mites (HDMs), and at 13 years levels of IgEs to peanuts, HDMs and mugwort were measured. IgE sensitization was considered positive if the specific IgE level was ≥0.35 kU/l.

Skin-prick tests (SPTs)

Forearm SPTs were carried out with commercial solutions (ALK-Abello Hørsholm, Denmark, or Stallergenes, Antony, France) or with fresh food preparations diluted with 0.9% saline. At six months, SPTs for egg allergens, at two years SPTs for egg, milk, fish, wheat, timothy grass, birch, and cat and dog allergens, and at five years SPTs for the same allergens as at two years but also for peanuts, mugwort and HDMs were carried out. A wheal diameter of 3 mm or more than the negative control was considered positive. We used the term “any specific SPT ≥3 mm” to indicate that any particular one of the specific SPT results was ≥3 mm in diameter.

Statistical analysis

Chi-square and Fisher’s exact tests were used to determine the effects of probiotics on children´s sensitization. Potential confounders considered were sex, study group, breast feeding for ≥6 months, parental education, parental atopy, mode of delivery, parental smoking, siblings at birth, use of antibiotics during the first three months, use of other products containing probiotics during the first three months by mother or child, and cat/dog at home for the first three months. We used cesarean/vaginal delivery, maternal atopy and exclusive breastfeeding over three months as interaction terms. The outcomes concerning potential confounders were adjusted by using multivariate logistic regression analysis; results are given as odd ratios with 95% confidence intervals (Cls). $p<0.05$ was considered statistically significant. Our outcome measures were the prevalence rates of sensitization to any allergen, any food, any animal and any pollen in the probiotic and placebo groups at two, five and 13 years. Analyses were performed with IBM-SPSS software, v. 22.

4. Results:

Total cohort

Blood samples were available from 684 (67%), 737 (72%) and 459 (45%) children at two, five and 13 years among those who originally met the inclusion criteria and were intended to be treated. Baseline characteristics of the participants were comparable in the probiotic and placebo groups at these time points (data not shown). In the probiotic and placebo groups, 74% and 74% at two years, 84% and 81% at five years and 75% and 68% at 13 years among those who completed follow-up, gave blood samples (Fig.1).

Sensitization in the probiotic and placebo groups

The prevalence rates of allergic sensitization (any specific IgE ≥0.35 kU/l and/or any specific SPT ≥3 mm) were similar in the probiotic and placebo groups at two (40.2% vs. 43.3%) and five years (55.3%

vs. 53.5%) (Table 1). No specific differences were found in SPT positivity between the two groups at two and five years

(Table 2). The prevalence rates of IgE sensitization (any specific IgE ≥0.35 kU/l) were similar in the probiotic and placebo groups at every time point (Table 1). When comparing the rates of sensitization we found that the prevalence of IgE sensitization (any specific IgE ≥0.35 kU/l) was higher than the prevalence of SPT positivity (any specific SPT ≥3 mm) in the probiotic (31.1% vs. 22.6% and 50.1% vs. 40.3%) and in the placebo groups (34.1% vs. 25.2% and 45.6% vs. 40.4%) at two and five years (Tables 1 and 2).

No significant differences were found in the prevalence of IgE sensitization to egg and/or milk at two (22.7% vs. 27.0%), five (28.3% vs. 30.4%) or 13 years (9.8% vs. 9.9%) in the two groups. IgE sensitization to birch and/or timothy grass was also similar at two (14.2% vs. 12.3%), five (34.7% vs. 32.0%) and 13 years (51.6% vs. 51.2%). The prevalence rates of IgE sensitization to cat and/or dog dander at two (6.2% vs. 6.4%) and five years (16.8% vs. 15.2%) were comparable in the two groups. At 13 years the prevalence of IgE sensitization to cat and/or dog dander was higher in the probiotic group compared with the placebo group (40.2% vs. 31.0%; adjusted OR 0.63; 95% CI 0.42–0.96; p=0.03). The difference was seen in the vaginallydelivered children (40.4% vs. 28.3%; adjusted OR 0.56; 95% CI 0.35–0.90; p=0.02), but not in the cesareandelivered group (39.5% vs. 42.5%) at 13 years. In the subgroup of vaginally-delivered children the prevalence of IgE sensitization to birch and/or timothy grass was higher in the probiotic group than in the placebo group (15.4% vs. 10.7%, adjusted OR 0.51; 95% CI 0.30–0.88; p=0.02) at two years, with no significant difference at five years (33.2% vs. 32.1%) or 13 years (52.4% vs. 49.1%). In the subgroup of children who were exclusively breastfed for more than three months no significant difference was detected in the prevalence of sensitization (any specific IgE ≥0.35 kU/l and/or any specific SPT ≥3 mm) between the probiotic and placebo groups at two years (41.2% vs. 41.6%) or at five years (52.2% vs. 53.3%). No difference was found between the two groups in the prevalence of sensitization (any specific IgE ≥0.35 kU/l and/or any specific SPT ≥3 mm) in the subgroup of children with atopic mothers at two years (39.5% vs. 46.2%) or at five years (55.8% vs. 54.7%). Nevertheless, in the subgroup of children with an atopic mother the prevalence of IgE sensitization to cat and/or dog dander was higher in the probiotic group compared with the placebo group (42.4% vs. 32.6%; adjusted OR 0.59; 95% CI 0.37–0.94; p=0.03) at 13 years (Table 1).

5. Discussion:

We found no difference in sensitization to any allergen between the probiotic (LGG, *L. rhamnosus*, *B. breve* and *Propionibacterium freudenreichii* ssp. *shermanii*) and placebo groups at two, five or 13 years in high-risk children. The results are similar to those in several previous studies (1, 4, 11). Unlike in our study, SPT-confirmed sensitization (birch, cat dander, milk, dog dander, egg, HDM, peanut and timothy grass) has been reported to be less common in a probiotic (*L. reuteri*) group than in a placebo group at two years, but only in children with allergic mothers. However, no such effect was seen at seven years (5). In addition, the prevalence of SPT positivity (cat dander, milk, egg, HDM, mixed grass, peanut) has been reported to be lower in a probiotic (*L. rhamnosus*) group than in a placebo group for up to six years (6).

In contrast to studies showing lower rates of sensitization to common allergens in probiotic groups than in placebo groups (5, 6, 9), we found that IgE sensitization to birch and/or timothy grass at two years was similar in both groups in the total cohort, but, surprisingly, IgE sensitization to birch and/or timothy grass at 13 years was more common in the probiotic group than in the placebo group in the subgroup of children delivered vaginally. Whereas sensitization to birch/timothy was similar in the probiotic and placebo groups at two, five and 13 years, as was sensitization to cat/dog dander at two and five years, it was significantly higher in the probiotic group at 13 years in the total cohort. The effects of probiotics on allergic sensitization seem to be species-specific, as has been shown for development of allergic disease (5, 6, 9, 11).

Sensitization patterns and immunologic reactivity are different as regards perennial and seasonal allergens and they may also be differently affected by environmental and dietary differences such as probiotic supplementation (12, 13). Supplementation with *L. acidophilus* six months after delivery has been associated with an elevated level of SPT-verified sensitization (cat dander, milk, grass, HDM, mold, peanut and egg) compared with placebo at one year (14), while in the same cohort at five years there was no significant difference (15). Whether this reflects true effects of probiotics or normal variability in the development of IgE sensitization should tested in more studies designed to investigate the long-term development of sensitization.

We know there are some risk factors of IgE sensitization such as atopic eczema (16), but the development of sensitization and clinical allergic disease seem to occur in different phenotypes, and the immunologic basis of this is largely unknown. While the pattern of sensitization for each allergen is different, allergic sensitization to egg and cats are risk factors of all allergic diseases (17). There are several risk/protective factors associated with allergic disease and/or sensitization. Some elements are associated with both allergic disease and sensitization; others affect only one or the other and results are controversial. For example, results concerning breastfeeding and the risks of sensitization and allergic diseases are contentious, with no association between long durations of breastfeeding, and sensitization or allergic disease in high-risk patients (18). These results are in line with ours in that no difference was found in sensitization between the probiotic and placebo groups in the subgroup of children who were breastfed for more than three months. However, in a population-based study ≥4 months of breastfeeding diminished the risk of childhood eczema at four years (19) as well as the risks of asthma and sensitization at eight years (20).

A strength of our study is that compliance was relatively high, as 45% of the participants gave blood samples at 13 years, and 67–72% at two and five years, and another strength of the study is the long follow-up period of 13 years. The main limitations of the study design are the lack of skin-prick tests and pediatric examination at the age of 13 years.

The prevalence of sensitization to any common allergen was comparable in the probiotic and placebo groups up to the age of 13 years. At 13 years, IgE sensitization to cat and/or dog dander was more common in the probiotic group than in the placebo group. In the subgroup of vaginally delivered children, IgE sensitization to birch and/or timothy grass was also more common in the probiotic group than in the placebo group at two years. Our results indicate that a mixture of pro- and prebiotics used perinatally might still affect IgE sensitization to aeroallergens at 13 years in high-risk children.

6. Appendix: -

7. Supplementary Material: -

8. Statements:

8.1 Acknowledgments: We thank Nick Bolton for language editing**.**

8.2 Statement of Ethics: Subjects (or their parents or guardians) gave written informed consent. The study protocol was approved by the research institute's committee on human research. The clinical trials registration number is NCT00298337. The Ethics Committee of the Hospital for Children and Adolescents, Helsinki University, approved the study.

8.3 Disclosure Statement: The authors have no conflicts of interest to declare.

8.4 Funding sources: none

8.5 Author contributions:

Savilahti, Kukkonen and Kuitunen designed the study and they also collected the data. Peldan analyzed the data, designed the figures and wrote the manuscript. All authors provided critical feedback and helped shape the analysis and manuscript.

9. References:

References

1. 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 Apr;119(4):101921.

2. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. J Allergy Clin Immunol. 2007 Jan;119(1):192-8.

3. 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 Feb;123(2):335-41.

4. Rautava S, Kainonen E, Salminen S, Isolauri E. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. J Allergy Clin Immunol. 2012 Dec;130(6):1355-60.

5. Abrahamsson TR, Jakobsson T, Bjorksten B, Oldaeus G, Jenmalm MC. No effect of probiotics on respiratory allergies: a seven-year follow-up of a randomized controlled trial in infancy. Pediatr Allergy Immunol. 2013 Sep;24(6):556-61.

6. Wickens K, Stanley TV, Mitchell EA, Barthow C, Fitzharris P, Purdie G, et al. Early supplementation with Lactobacillus rhamnosus HN001 reduces eczema prevalence to 6 years: does it also reduce atopic sensitization? Clin Exp Allergy. 2013 Sep;43(9):1048-57.

7. Taylor AL, Hale J, Wiltschut J, Lehmann H, Dunstan JA, Prescott SL. Effects of probiotic supplementation for the first 6 months of life on allergen- and vaccine-specific immune responses. Clin Exp Allergy. 2006 Oct;36(10):1227-35.

8. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. Pediatrics. 2008 Apr;121(4):e850-6.

9. Allen SJ, Jordan S, Storey M, Thornton CA, Gravenor MB, Garaiova I, et al. Probiotics in the prevention of eczema: a randomised controlled trial. Arch Dis Child. 2014 Nov;99(11):1014-9.

10. Peldan P, Kukkonen AK, Savilahti E, Kuitunen M. Perinatal probiotics decreased eczema up to 10 years of age, but at 5-10 years, allergic rhino-conjunctivitis was increased. Clin Exp Allergy. 2017 Jul;47(7):975-9.

11. 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 Oct;122(4):788-94.

12. Smith KA, Gray NJ, Saleh F, Cheek E, Frew AJ, Kern F, et al. Characterisation of CD154+ T cells following ex vivo allergen stimulation illustrates distinct T cell responses to seasonal and perennial allergens in allergic and non-allergic individuals. BMC Immunol. 2013 Nov 5;14:49,2172-14-49.

13. Westman M, Asarnoj A, Hamsten C, Wickman M, van Hage M. Windows of opportunity for tolerance induction for allergy by studying the evolution of allergic sensitization in birth cohorts. Semin Immunol. 2017 Apr;30:61-6.

14. 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 Jan;119(1):184-91.

15. Jensen MP, Meldrum S, Taylor AL, Dunstan JA, Prescott SL. Early probiotic supplementation for allergy prevention: long-term outcomes. J Allergy Clin Immunol. 2012 Nov;130(5):1209,1211.e5.

16. Lack G, Fox D, Northstone K, Golding J, Avon Longitudinal Study of Parents and Children Study Team. Factors associated with the development of peanut allergy in childhood. N Engl J Med. 2003 Mar 13;348(11):977-85.

17. Ronmark E, Perzanowski M, Platts-Mills T, Lundback B. Different sensitization profile for asthma, rhinitis, and eczema among 7-8-year-old children: report from the Obstructive Lung Disease in Northern Sweden studies. Pediatr Allergy Immunol. 2003 Apr;14(2):91-9.

18. Jelding-Dannemand E, Malby Schoos AM, Bisgaard H. Breast-feeding does not protect against allergic sensitization in early childhood and allergy-associated disease at age 7 years. J Allergy Clin Immunol. 2015 Nov;136(5):1302,8.e1-13.

19. Kull I, Bohme M, Wahlgren CF, Nordvall L, Pershagen G, Wickman M. Breast-feeding reduces the risk for childhood eczema. J Allergy Clin Immunol. 2005 Sep;116(3):657-61.

20. Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, et al. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. J Allergy Clin Immunol. 2010 May;125(5):1013-9.

10. Figure legends:

Fig.1. Flow of participants in the probiotic and placebo groups

The clinical trials registration number is NCT00298337.

Table 1. Sensitization in the probiotic and placebo groups

Sensitization	Probiotic, *n* (%)	Placebo, *n* (%)	Unadjusted OR (95% CI)	*p* value*	Adjusted OR (95% CI)	Adjusted *p* value***
Total cohort						
2 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	148/368 (40.2)	161/372 (43.3)	0.88 (0.66–1.18)	0.398	1.06 (0.77–1.46)	0.705
Food (egg and/or milk) IgE ≥0.35 kU/L	77/339 (22.7)	93/344 (27.0)	0.79 (0.56–1.12)	0.192	1.13 (0.78–1.64)	0.520
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	48/337 (14.2)	42/342 (12.3)	1.19 (0.76–1.85)	0.451	0.71 (0.44–1.16)	0.172
Animal (cat and/or dog) IgE ≥0.35 kU/L	21/338 (6.2)	22/343 (6.4)	0.97 (0.52–1.79)	0.914	1.07 (0.54–2.11)	0.848
Any specific IgE ≥0.35 kU/L	105/338 (31.1)	117/343 (34.1)	0.87 (0.63–1.20)	0.397	1.04 (0.74–1.48)	0.814
5 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	214/387 (55.3)	209/391 (53.5)	1.08 (0.81–1.43)	0.606	1.07 (0.75–1.54)	0.713
Food (egg and/or milk) IgE ≥0.35 kU/L	106/375 (28.3)	110/362 (30.4)	0.90 (0.66–1.24)	0.527	1.49 (0.95–2.35)	0.084
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	130/375 (34.7)	116/362 (32.0)	1.13 (0.83–1.53)	0.450	0.96 (0.64–1.44)	0.849
Animal (cat and/or dog) IgE ≥0.35 kU/L	63/375 (16.8)	55/362 (15.2)	1.13 (0.76–1.67)	0.552	1.29 (0.74–2.25)	0.377
Any specific IgE ≥0.35 kU/L	188/375 (50.1)	165/362 (45.6)	1.20 (0.90–1.60)	0.216	0.98 (0.67–1.42)	0.896
13 years						
Food (egg and/or milk) IgE ≥0.35 kU/L	24/246 (9.8)	21/213 (9.9)	0.99 (0.53–1.83)	0.970	0.99 (0.52–1.88)	0.966
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	127/246 (51.6)	109/213 (51.2)	1.02 (0.71–1.47)	0.923	0.99 (0.66–1.49)	0.960
Animal (cat and/or dog) IgE ≥0.35 kU/L	99/246 (40.2)	66/213 (31.0)	1.50 (1.02–2.21)	0.039	0.63 (0.42–0.96)	0.030
Any specific IgE ≥0.35 kU/L	151/246 (61.4)	121/213 (56.8)	1.21 (0.83–1.76)	0.320	0.78 (0.52–1.19)	0.249
Cesarean delivery						
2 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	21/62 (33.9)	31/64 (48.4)	0.55 (0.27–1.12)	0.097	1.63 (0.72–3.67)	0.240
Food (egg and/or milk) IgE ≥0.35 kU/L	12/57 (21.1)	19/62 (30.2)	0.62 (0.27–1.42)	0.255	1.48 (0.61–3.62)	0.387
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	5/57 (8.8)	12/62 (19.4)	0.40 (0.13–1.22)	0.099	2.03 (0.61–6.84)	0.251
Animal (cat and/or dog) IgE ≥0.35 kU/L	4/57 (7.0)	3/62 (4.8)	1.48 (0.32–6.94)	0.709**	0.80 (0.14–4.63)	0.806
Any specific IgE ≥0.35 kU/L	14/57 (24.6)	27/62 (43.5)	0.42 (0.19–0.93)	0.029	2.07 (0.90–4.77)	0.087
5 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	27/54 (50.0)	38/73 (52.1)	0.92 (0.46–1.86)	0.819	1.96 (0.64–6.03)	0.242
Food (egg and/or milk) IgE ≥0.35 kU/L	17/53 (32.1)	11/66 (16.7)	2.36 (0.99–5.62)	0.049	0.69 (0.11–4.33)	0.688
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	20/53 (37.7)	21/66 (31.8)	1.30 (0.61–2.76)	0.500	2.89 (0.76–10.77)	0.114
Animal (cat and/or dog) IgE ≥0.35 kU/L	8/53 (15.1)	13/66 (19.7)	0.73 (0.28–1.91)	0.513	2.96 (0.55–16.04)	0.208
Any specific IgE ≥0.35 kU/L	26/53 (49.1)	25/66 (37.9)	1.58 (0.76–3.29)	0.221	1.23 (0.38–3.97)	0.731
13 years						
Food (egg and/or milk) IgE ≥0.35 kU/L	6/38 (15.8)	5/40 (12.5)	1.31 (0.37–4.72)	0.677	0.51 (0.12–2.22)	0.370
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	18/38 (47.4)	24/40 (60.0)	0.60 (0.25–1.47)	0.263	2.36 (0.73–7.67)	0.152
Animal (cat and/or dog) IgE ≥0.35 kU/L	15/38 (39.5)	17/40 (42.5)	0.88 (0.36–2.18)	0.786	0.88 (0.30–2.59)	0.821
Any specific IgE ≥0.35 kU/L	23/38 (60.5)	27/40 (67.5)	0.74 (0.29–1.87)	0.521	0.88 (0.30–2.59)	0.821
Vaginal delivery						
2 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	127/306 (41.5)	130/308 (42.2)	0.97 (0.71–1.34)	0.860	0.94 (0.66–1.34)	0.742
Food (egg and/or milk) IgE ≥0.35 kU/L	65/282 (23.0)	74/281 (26.3)	0.84 (0.57–1.23)	0.366	1.10 (0.73–1.65)	0.657
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	43/280 (15.4)	30/280 (10.7)	1.51 (0.92–2.49)	0.103	0.51 (0.30–0.88)	0.016
Animal (cat and/or dog) IgE ≥0.35 kU/L	17/281 (6.0)	19/281 (6.8)	0.89 (0.45–1.75)	0.730	1.08 (0.52–2.25)	0.835
Any specific IgE ≥0.35 kU/L	91/281 (32.4)	90/281 (32.0)	1.02 (0.71–1.45)	0.928	0.86 (0.59–1.26)	0.442
5 years						
Any SPT pos. and/or any IgE ≥0.35 kU/L	187/333 (56.2)	171/318 (53.8)	1.10 (0.81–1.50)	0.541	0.99 (0.67–1.47)	0.970
Food (egg and/or milk) IgE ≥0.35 kU/L	89/322 (27.6)	99/296 (33.4)	0.76 (0.54–1.07)	0.117	1.54 (0.95–2.49)	0.081
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	110/322 (33.2)	95/296 (32.1)	1.10 (0.79–1.54)	0.586	0.91 (0.59–1.41)	0.683
Animal (cat and/or dog) IgE ≥0.35 kU/L	55/322 (17.1)	42/296 (14.2)	1.25 (0.81–1.93)	0.324	1.15 (0.62–2.12)	0.665
Any specific IgE ≥0.35 kU/L	162/322 (50.3)	140/296 (47.3)	1.13 (0.82–1.55)	0.454	0.98 (0.65–1.47)	0.904
13 years						
Food (egg and/or milk) IgE ≥0.35 kU/L	18/208 (8.7)	16/173 (9.2)	0.93 (0.46–1.88)	0.839	1.06 (0.49–2.26)	0.891
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	109/208 (52.4)	85/173 (49.1)	1.14 (0.76–1.71)	0.544	0.87 (0.55–1.37)	0.537
Animal (cat and/or dog) IgE ≥0.35 kU/L	84/208 (40.4)	49/173 (28.3)	1.71 (1.11–2.64)	0.017	0.56 (0.35–0.90)	0.019
Any specific IgE ≥0.35 kU/L	128/208 (61.5)	94/173 (54.3)	1.35 (0.89–2.03)	0.156	0.71 (0.45–1.12)	0.137

Table 1 (continued)

Sensitization	Probiotic, *n* (%)	Placebo, *n* (%)	Unadjusted OR (95% CI)	*p* value*	Adjusted OR (95% CI)	Adjusted *p* value***
Maternal atopy						
2 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	120/304 (39.5)	138/299 (46.2)	0.76 (0.55–1.05)	0.097	1.21 (0.85–1.72)	0.285
Food (egg and/or milk) IgE ≥0.35 kU/L	60/281 (21.4)	84/281 (29.9)	0.64 (0.43–0.93)	0.02	1.48 (0.98–2.23)	0.061
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	38/279 (13.6)	36/279 (12.9)	1.06 (0.65–1.74)	0.803	0.76 (0.44–1.30)	0.314
Animal (cat and/or dog) IgE ≥0.35 kU/L	17/280 (6.1)	20/280 (7.1)	0.84 (0.43–1.64)	0.610	1.18 (0.56–2.47)	0.662
Any specific IgE ≥0.35 kU/L	84/280 (30.0)	104/280 (37.1)	0.73 (0.51–1.03)	0.074	1.27 (0.87–1.86)	0.211
5 years						
Any positive SPT and/or any IgE ≥0.35 kU/L	179/321 (55.8)	175/320 (54.7)	1.04 (0.77–1.43)	0.784	1.14 (0.76–1.70)	0.528
Food (egg and/or milk) IgE ≥0.35 kU/L	88/309 (28.5)	96/300 (32.0)	0.85 (0.60–1.20)	0.344	1.58 (0.95–2.61)	0.075
Pollen (birch and/or timothy) IgE ≥0.35 kU/L	104/309 (33.7)	98/300 (32.7)	1.05 (0.75–1.47)	0.795	1.02 (0.65–1.59)	0.945
Animal (cat and/or dog) IgE ≥0.35 kU/L	56/309 (18.1)	46/300 (15.3)	1.22 (0.80–1.87)	0.357	1.02 (0.56–1.86)	0.948
Any specific IgE ≥0.35 kU/L	156/309 (50.5)	142/300 (47.3)	1.13 (0.83–1.56)	0.437	1.06 (0.70–1.61)	0.781
13 years						
Food (egg and/or milk) IgE ≥0.35 kU/L	21/203 (10.3)	17/172 (9.9)	1.05 (0.54–2.07)	0.883	0.88 (0.42–1.81)	0.719
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	108/203 (53.2)	91/172 (52.9)	1.01 (0.67–1.52)	0.955	0.93 (0.59–1.48)	0.764
Animal (cat and/or dog) IgE ≥0.35 kU/L	86/203 (42.4)	56/172 (32.6)	1.52 (1.00–2.33)	0.051	0.59 (0.37–0.94)	0.026
Any specific IgE ≥0.35 kU/L	133/203 (65.5)	103/172 (59.9)	1.27 (0.84–1.94)	0.260	0.73 (0.46–1.17)	0.194
Breastfeeding for ≥3 months						
2 years						
Any SPT pos. and/or any IgE ≥0.35 kU/L	73/177 (41.2)	72/173 (41.6)	0.99 (0.64–1.51)	0.943	1.01 (0.64–1.61)	0.958
Food (egg and/or milk) IgE ≥0.35 kU/L	41/170 (24.1)	45/158 (28.5)	0.80 (0.49–1.31)	0.369	1.14 (0.67–1.95)	0.631
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	26/168 (15.5)	18/158 (11.4)	1.42 (0.75–2.71)	0.281	0.72 (0.36–1.46)	0.366
Animal (cat and/or dog) IgE ≥0.35 kU/L	11/169 (6.5)	7/158 (4.4)	1.50 (0.57–3.98)	0.410	0.86 (0.29–2.61)	0.793
Any specific IgE ≥0.35 kU/L	56/169 (33.1)	52/158 (32.9)	1.01 (0.64–1.60)	0.966	0.96 (0.58–1.58)	0.858
5 years						
Any SPT pos. and/or any IgE ≥0.35 kU/L	97/186 (52.2)	89/167 (53.3)	0.96 (0.63–1.45)	0.830	1.16 (0.67–2.01)	0.587
Food (egg and/or milk) IgE ≥0.35 kU/L	45/180 (25.0)	46/157 (29.3)	0.80 (0.50–1.30)	0.375	1.54 (0.73–3.28)	0.260
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	60/180 (33.3)	51/157 (32.5)	1.04 (0.66–1.64)	0.869	0.98 (0.53–1.83)	0.951
Animal (cat and/or dog) IgE ≥0.35 kU/L	23/180 (12.8)	25/157 (15.9)	0.77 (0.42–1.43)	0.410	1.99 (0.81–4.93)	0.136
Any specific IgE ≥ 0.35kU/L	84/180 (46.7)	71/157 (45.2)	1.06 (0.69–1.63)	0.791	0.97 (0.54–1.76)	0.927
13 years						
Food (egg and/or milk) IgE ≥0.35 kU/L	14/130 (10.8)	7/101 (6.9)	1.62 (0.63–4.18)	0.314	0.50 (0.18–1.38)	0.183
Pollen (birch and/or Timothy) IgE ≥0.35 kU/L	66/130 (50.8)	48/101 (47.5)	1.14 (0.68–1.92)	0.625	0.99 (0.55–1.79)	0.981
Animal (cat and/or dog) IgE ≥0.35 kU/L	48/130 (36.9)	29/101 (28.7)	1.45 (0.83–2.54)	0.189	0.62 (0.34–1.14)	0.124
Any specific IgE ≥0.35 kU/L	82/130 (63.8)	55/101 (54.5)	1.48 (0.87–2.51)	0.149	0.71 (0.39–1.27)	0.245

* *p* value (χ^2 test) for differences between 2 study groups. ** *p* value (Fisher's exact test) for differences between 2 study groups. *** Adjusted by means of multivariate logistic regression for sex, study group, breastfeeding for ≥6 months, university-educated parent, parental atopy, mode of delivery, parental smoking, siblings at the time of birth, use of antibiotics during the first 3 months, use of other products containing probiotics during the first 3 months by the mother or the child, and cat/dog at home for the first 3 months. Cesarean delivery/vaginal delivery/maternal atopy/breastfeeding for ≥3 months were used as interaction terms.

Table 2. SPT positivity in the probiotic and placebo groups

Positive SPT	Probiotic, *n* (%)	Placebo, *n* (%)	Unadjusted OR (95% CI)	*p* value*	Adjusted OR (95% CI)	Adjusted *p* value***
2 years						
Egg	58/453 (12.8)	70/460 (15.2)	0.82 (0.56–1.19)	0.294	1.20 (0.79–1.82)	0.390
Milk	12/453 (2.6)	15/461 (3.3)	0.81 (0.37–1.75)	0.589	1.25 (0.55–2.85)	0.595
Wheat	12/452 (2.7)	9/461 (2.9)	1.37 (0.57–3.28)	0.479	0.52 (0.20–1.37)	0.187
Fish	4/452 (0.9)	2/460 (0.4)	2.05 (0.37–11.22)	0.488**	0.45 (0.08–2.63)	0.378
Birch	44/452 (9.7)	47/460 (10.2)	0.05 (0.61–1.46)	0.808	0.91 (0.58–1.45)	0.701
Timothy	3/452 (0.7)	2/461 (0.4)	1.53 (0.26–9.22)	0.684**	0.57 (0.09–3.75)	0.561
Cat	24/453 (5.3)	18/461 (3.9)	1.38 (0.74–2.57)	0.314	0.77 (0.39–1.52)	0.449
Dog	24/453 (5.3)	21/460 (4.6)	1.17 (0.64–2.13)	0.609	0.92 (0.47–1.78)	0.796
Any of the following: egg, milk, birch, Timothy, cat, dog	102/452 (22.6)	116/460 (25.2)	0.86 (0.64–1.17)	0.348	1.12 (0.81–1.57)	0.495
5 years						
Egg	29/387 (7.5)	35/391 (9.0)	0.82 (0.49–1.38)	0.459	2.02 (0.89–4.62)	0.094
Milk	6/387 (1.6)	7/391 (1.8)	0.86 (0.29–2.59)	0.794	1.10 (0.26–4.67)	0.900
Wheat	12/387 (3.1)	10/391 (2.6)	1.22 (0.52–2.86)	0.648	0.76 (0.19–3.04)	0.698
Fish	11/387 (2.8)	3/391 (0.8)	3.78 (1.05–13.67)	0.029	0.25 (0.05–1.24)	0.090
Birch	122/387 (31.5)	124/391 (31.7)	0.99 (0.73–1.34)	0.955	1.25 (0.84–1.84)	0.269
Timothy	52/387 (13.4)	52/391 (13.3)	1.01 (0.67–1.53)	0.955	0.86 (0.51–1.45)	0.571
Cat	51/387 (13.2)	57/391 (14.6)	0.89 (0.59–1.34)	0.572	1.54 (0.87–2.71)	0.138
Dog	45/387 (11.6)	49/391 (12.5)	0.92 (0.60–1.41)	0.699	1.03 (0.56–1.89)	0.916
House dust mites	5/387 (1.3)	4/391 (1.0)	1.27 (0.34–4.75)	0.751**	0.35 (0.07–1.74)	0.197
Mugwort	16/387 (4.1)	22/391 (5.6)	0.72 (0.37–1.40)	0.344	1.08 (0.44–2.63)	0.867
Peanut	51/387 (13.2)	52/391 (13.3)	0.99 (0.65–1.50)	0.960	0.99 (0.58–1.72)	0.981
Any of the following: egg, milk, birch, Timothy, cat, dog	156/387 (40.3)	158/391 (40.4)	1.00 (0.75–1.33)	0.978	1.20 (0.83–1.73)	0.339

* *p* value (χ^2 test) for differences between 2 study groups. ** *p* value (Fisher's exact test) for differences between 2 study groups. *** Adjusted by means of multivariate logistic regression for sex, study group, breastfeeding for ≥6 months, university-educated parent, parental atopy, mode of delivery, parental smoking, siblings at the time of birth, use of antibiotics during the first 3 months, use of other products containing probiotics during the first 3 months by the mother or the child, and cat/dog at home for the first 3 months.

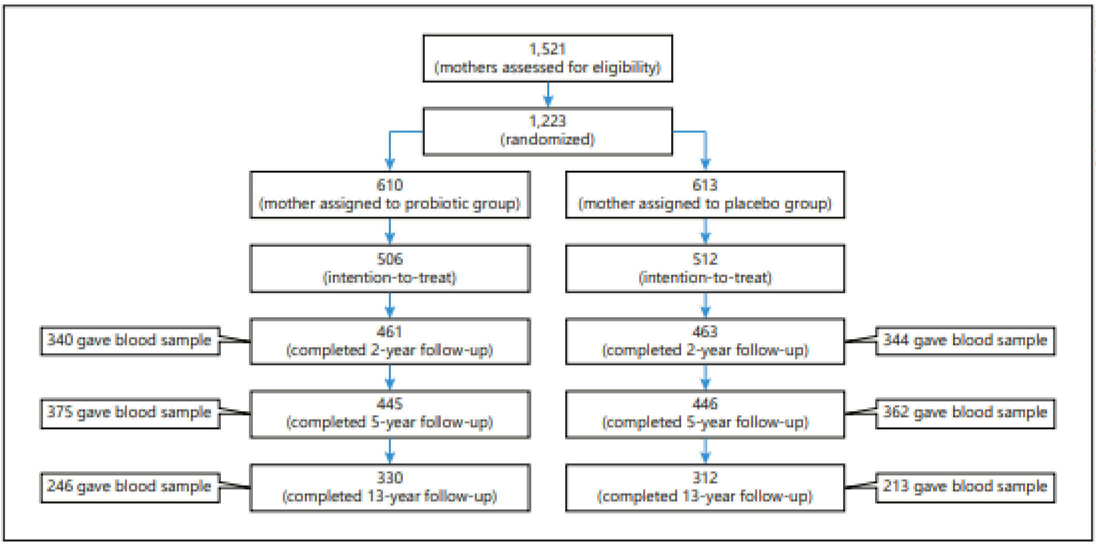

Fig. 1. Flow of participants in the probiotic and placebo groups.