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# Perinatal probiotic intervention prevented allergic disease in a Caesarean-delivered subgroup at 13-year follow-up

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**Abstract**

Background: The long-term effects of probiotic intervention for primary prevention of allergic diseases are not well known. We previously reported less eczema until 10 years in our probiotic intervention trial.

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Objective: To investigate the effect of early probiotic intervention on the prevalence of allergic diseases up to 13 years of age.

Methods: Pregnant women (n=1223) carrying a child at a high risk of allergy (at least one parent with allergic disease) were randomised to receive a mixture of probiotics (*Lactobacillus rhamnosus* GG and LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii*) or placebo in a double-blind manner from 36 weeks of gestation until birth. Their infants received the same product for the first six months (registration number NCT00298337). At 13-year follow-up the participants were requested to return a questionnaire and to provide a blood sample.

Results: A questionnaire was returned by 642 participants (63.1% of intention-to-treat infants) and 459 provided a blood sample. In the whole cohort there were no statistically significant differences in doctor-diagnosed allergic disease (55.2% and 59.0%, probiotic and placebo group, respectively) or allergic disease (47.9% and 51.6%) based on the ISAAC questionnaire data. Inhalant-specific IgE-sensitisation (>0.7 kU/L) was 59.3% in the probiotic group and 49.8% in the placebo group (p=0.040). In a post hoc analysis made in Caesarean-delivered subgroup allergy was reported in 41.5% of the probiotic group and 67.9% of the placebo group (p=0.006), and eczema in 18.9% and 37.5% respectively (p=0.031). In the whole cohort 8.5% of the probiotic group had suffered from wheezing attacks during the previous 12 months vs. 14.7% in the placebo group (p=0.013). There was no statistically significant differences discovered between the characteristics of the participating group and the dropout-group.

Conclusions: Probiotic intervention protected Caesarean-delivered subgroup from allergic disease and eczema, but not the total cohort.

**Introduction**

The prevalence of allergic disease has increased and is especially a burden for the child population (1). The increase in prevalence is connected to westernization and is believed to be connected to decreased microbial exposure. Living on a farm (2), and consuming farm milk (3) has been linked to fewer allergic diseases, which might be associated with greater microbial exposure. Birth via

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Caesarean section is connected to higher risk of asthma (4,5). A connection between early-life microbiota composition and atopy has been reported (6,7), and non-allergic children's microflora has been shown to be more diverse (8). The presence of probiotic bacteria in the intestinal microflora during the first year of life is connected to less allergic morbidity up to two years of age (9).

Probiotics are defined as 'live microorganisms which when administered in adequate amounts, confer a health benefit on the host' (Food and Agricultural Organisation). In a large observational cohort study of over 40 000 participants, consumption of probiotic milk products in pregnancy and infancy was associated with a slightly reduced risk of atopic eczema at six months and reduced rhinoconjunctivitis at 18 to 36 months. There was no association with asthma. The information about the probiotic usage was based on self-report. The probiotics used were three dairy products, Biola milk, Biola yogurt and Cultura milk (10), which contained *L. acidophilus* (LA-5).

It has been shown in several studies that probiotics given pre- and postnatally prevent eczema for up to two years of age (11–13). In longer 6-year follow-up studies an allergy-reducing effect has been reported in some (14,15) but not all studies (16). Kalliomäki et al. reported less atopic eczema for up to seven years (17). Other 7- to 8-year follow-up studies have shown no effect (18,19). In previous phases of our study, the largest probiotic intervention trial reported to date, we found 29% less atopic disease at two years of age in the group receiving probiotics in the perinatal period (20). In the 5-year follow-up there was no statistically significant difference in the whole cohort, but the Caesarean-delivered children in the probiotic group had significantly less IgE-associated allergy (21). At 10 years of age the reducing effect on eczema was still visible and, surprisingly, allergic rhinoconjunctivitis was increased at 5–10 years (22) . The objective of this follow-up was to investigate the effect of perinatal probiotic intervention on the prevalence of allergic diseases and sensitisation at 13-years of age.

**Methods**

Pregnant women carrying a child at a high risk of allergy were recruited for the trial. The criterion for high allergy risk was that at least one of the parents had had doctor-diagnosed asthma, allergic rhinitis, or atopic eczema. The participants (n=1223) were randomised to receive a preparation of

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four probiotics or placebo in a double-blind manner (Chart 1). Starting from 36 weeks of gestation mothers in the probiotic group received one capsule containing freeze-dried LGG (American Type Culture Collection 53103; $5 \times 10^9$ colony-forming units (CFU)), *L. rhamnosus* LC705 (DSM 7061; $5 \times 10^9$ CFU), *Bifidobacterium breve* Bb99 (DSM 13692; $2 \times 10^8$ CFU) and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7076; $2 \times 10^9$ CFU) twice a day until delivery. Their infants received the same probiotic capsule opened and mixed with 20 drops of syrup containing 0.8 g of galacto-oligosaccharides once daily for the first six months of their life.

Mothers in the placebo group received capsules containing microcrystalline cellulose, and the infants received the contents of the same capsules, with no oligosaccharides. The capsules and syrups used in the trial looked, smelled and tasted identical. The viability of the bacteria was regularly controlled. Use of non-study probiotic products (Table 2) was not restricted after the intervention phase. Exclusion criteria were birth at less than 37 weeks of gestation, major malformations and the second-born of twins.

Compliance with the intervention was controlled at 3- and 6-month visits by questioning about the amount of doses not given and by counting the returned unused capsules. The 80% compliance level for the 180 days intervention was reached by 87% in the probiotic group and 88% in the placebo group. During their early years, the children were examined by a paediatrician at six months, two and five years and questionnaires concerning symptoms of allergic or infectious disease and related environmental factors were assessed annually up to five years (20). After the 5-year visit the participants parents and study staff were unblinded.

This 13-year follow-up study was carried out in 2013–15. A total of 945 previously reachable and willing participants received a letter asking them to complete a questionnaire on allergy, probiotic use and environmental factors. In the questionnaire we asked if the participants had had doctor-diagnosed eczema, asthma, rhinitis or food allergy, and there were more detailed questions about the symptoms, giving us more specific information on allergic morbidity in the preceding time, especially the previous 12 months. This questionnaire was based on the Finnish version of the International Study of Asthma and Allergies in Childhood (ISAAC) Questionnaire for 13- to 14-year-old children (23,24). The questionnaire includes questions about wheezing and coughing, sneezing, a runny or blocked nose, and eczema symptoms. Participants were asked to report the frequency of

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wheezing attacks over the past 12 months and the frequency of night wheezing in the questionnaire (Section 7.2 in the original questionnaire (25)). For the purpose of these analyses, wheezing attacks was dichotomised as "no attacks" and "one or more attacks" in the past 12 months. Night wheezing was dichotomised as “Less than one night a week” and “One or more nights per week”. Our research is not part of the ISAAC collaboration.

The primary outcome variable was doctor-diagnosed allergic disease (eczema, asthma, rhinitis, food allergy). A combination of questionnaire answers was used to define the presence of allergic illness during the previous 12 months. A symptom-report-based diagnosis of eczema was made if three specific questions were answered affirmatively, i.e. "Have you ever had an itchy rash which was coming and going for at least six months?", "Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, the front of the ankles, under the buttocks, or around the neck, ears, or eyes?" and "Have you had this itchy rash at any time in the past 12 months?". The child was considered to have had allergic rhinitis if the three following questions were answered affirmatively: "Have you ever had a problem with sneezing, or a runny or blocked nose when you DID NOT have a cold or the flu?", "In the past 12 months have you had a problem with sneezing, or a runny or blocked nose when you DID NOT have a cold or the flu?" and "In the past 12 months has this nose problem been accompanied by itchy-watery eyes?". The child was considered to have asthma if the two following questions were answered affirmatively: "Have you had wheezing or whistling in the chest in the past 12 months?" and "Have you ever had asthma?" Additionally, we recorded allergic disease combined with a serum antigen-specific IgE level of >0.7 kU/L. In the context of eczema and asthma, all specific IgE levels are considered. With rhinitis, only inhalant-specific IgE levels are considered, and with food allergy, only food-specific IgE levels are considered.

The participants were requested to come for appointment with research nurse, were blood samples were drawn to investigate IgE sensitisation status and to check essential haematological values for safety assessment of the probiotic intervention. Specific IgE antibodies were analysed – against birch, timothy grass, mugwort, cat dander, dog dander, house dust mites, egg white, milk and peanuts. Analysis was carried out using an ImmunoCAP system (Thermo Fisher Scientific Inc, Waltham, MA, USA). Haematological values analysed were haemoglobin concentration, red-blood cell indices and leucocyte and platelet counts. The research nurse also assessed the participants eczema using the SCORAD (SCORing Atopic Dermatitis) and measured height and weight.

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In a subgroup (n=296), peak expiratory flow (PEF) measurements were carried out to evaluate respiratory function. All participants were tested three times with reasonable resting time in between. Measurements were carried out with a hand-held Pinnacle Peak Flow Meter (Fyne Dynamics Ltd, Harlow, UK).

Pearson's chi-squared test was used to compare categorical variables. Continuous variables were compared with t-tests. An exploratory analysis was carried out to assess the effects of other potential predictive factors. These factors included sex, biparental atopy, mode of delivery, number of siblings, duration of total and exclusive breast feeding, parental education, smoking at home, household pets, antibiotic treatment during the intervention period and regular use of other probiotic preparations. The status of these factors was based on information gathered at 0 –2 years of age. Smoking at home and household pets were also considered at 13 years of age. Use of non-study probiotic preparations was considered at 0–2, 3–5 and 13 years. Interactions between the above predictive factors and the intervention were first assessed by using the Breslow–Day test. Doctor-diagnosed allergic disease, doctor-diagnosed allergic disease with sensitisation, ISAAC-based allergic disease and ISAAC-based allergic disease with sensitisation were used as an outcome variables for that analysis. Stratified analyses were subsequently performed for mode of delivery as statistically significant interactions were found between mode of delivery and these four outcomes (p-values for interactions were 0.860, 0.187, 0.009 and 0.025, respectively). Holm method was used to adjust for multiple comparisons. A separate analysis with missing values imputed from 10-year follow-up was also performed. All data analysis was carried out using IBM-SPSS® software version 24. The Ethics Committee of the Hospital for Children and Adolescents at Helsinki University Hospital approved the study. The clinical trials registration number for the trial is NCT00298337. There is no separate registration for the 13-year follow-up.

**Results**

In the initial phase of the study, there were 1018 intention-to-treat infants. A total of 642 participants (63.1%) completed the questionnaire at the 13-year follow-up, with 330 children from the probiotic group and 312 from the placebo group. The demographic characteristics of the groups were similar (Table 1). Among those who provided a blood sample (n = 459), 43.7 % had a household

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dog or cat at 13 years of age, compared to 32.9 % of the subgroup who did not provide a blood sample (p=0.010). There was no other significant differences between these two groups (Appendices, Table 6). In vaginally delivered subgroup 73.4% were breastfed for six months or more, compared to 63.3% in caesarean delivered subgroup (p=0.036). Mothers average age was 31.07 years in vaginally delivered and 32.57 years in caesarean delivered group (p=0.002). No other variables had significant differences between these two groups (Appendices, Table 6). Participants of the 13-year follow-up were compared to others in the intention-to-treat group. Infants, who dropped out in the early phase (64) had to be ignored in the analysis due to missing data. The others had no significant differences in the available variables compared to participants of the 13-year follow-up (Appendices, Table 6). The average age at the day of attending the study was 12.9 years, with only a 7-day difference between the probiotic and placebo groups. The prevalence of allergic disease at five years of age did not differ significantly between the cohort participating in the 13-year follow-up and those who did not participate (data not shown).

At 0–2 and 3–5 years of age the continuous consumption of non-study probiotics did not differ significantly between the probiotic and placebo groups (Table 2). In the 13-year follow-up, there was no significant difference in daily use of probiotics between the groups (9.7% in the probiotic group and 6.7% in the placebo group, p=0.172), but more participants in the probiotic group reported using probiotics weekly (16.1% vs. 10.6%, p=0.041) during the previous 12 months. These non-study products contained mainly *Lactobacillus rhamnosus* GG or *Lactobacillus reuteri*.

Among the primary clinical outcomes (Table 3), no statistically significant differences were found in the prevalence rates of doctor-diagnosed allergic disease (55.2% in the probiotic group and 59.0% in the placebo group, p=0.328) or any allergic disease with IgE sensitisation (42.7% and 39.9%, p=0.547). Regarding the secondary clinical outcomes (Table 3), no statistically significant differences between the groups were found in the prevalence rates of doctor-diagnosed asthma (12.7% and 17.0%, p=0.129), eczema (31.8% and 35.6%, p=0.314), food allergy (22.7% and 26.9%, p=0.218) or food-specific sensitisation (21.5% and 19.2%, p=0.543). However, the presence of inhalant-specific IgE-sensitisation (>0.7 kU/L) was 59.3% in the probiotic group and 49.8% in the placebo group (p=0.040). There were no differences in the prevalence rates of individual allergic diseases as assessed by use of the ISAAC questionnaire (Table 3).

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No significant differences between the probiotic and placebo groups were found as regards specific sensitisation to birch, timothy grass, mugwort, cat dander, dog dander, egg white, milk or peanuts (data not shown). The presence of a specific IgE to house dust mite was significantly lower in the probiotic group than in the placebo group (5.3% vs. 10.3%) at a >0.35 kU IgE/L level (Pearson's test, p=0.042 and Fisher's test, p=0.052). There was no difference at a >0.70 kU/L level (3.3% in the probiotic group and 5.6% in the placebo group, Pearson's test, p=0.213 and Fisher's test, p=0.255).

Among the primary clinical outcomes in the Caesarean-delivered subgroup (n=109), there were no significant differences in the lifetime prevalence rates of any doctor-diagnosed allergic disease (64.2% and 66.1%, p=0.833), nor any allergic disease with IgE sensitisation (39.5% and 50.0%, p=0.350) (Table 4). Regarding secondary clinical outcomes there were no statistically significant differences in doctor-diagnosed individual allergic diseases. The prevalence of any allergic disease in the previous 12 months (ISAAC) was 41.5% in the probiotic group and 67.9% in the placebo group (p=0.006). On the basis of responses to ISAAC questions, the prevalence of current eczema was less common in the probiotic group (18.9%) than in the placebo group (37.5%) (p=0.031). There were no significant differences in sensitisation between the probiotic and placebo groups.

Doctor-diagnosed allergic disease, doctor-diagnosed allergic disease with sensitisation, ISAAC-based allergic disease and ISAAC-based allergic disease with sensitisation for whole cohort and caesarean delivered subgroup are considered as the main outcome variables of the study. For the p-values of these 8 outcomes, Holm method was applied, and the difference in ISAAC-based allergic disease in caesarean delivered subgroup is considered significant. When the same analysis is applied on all p-values on tables 3 and 4, none of them are significant.

We also carried out an additional analysis for the primary and secondary clinical outcomes, where missing values where imputed from 10-year follow-up. Sensitisation was not analysed at 10-year follow-up, and sensitisation-related outcomes could not therefore be included in this analysis. There was no statistically significant results in the whole cohort (n=838). In the caesarean-delivered subgroup (n=144) the prevalence of any allergic disease in 12 months (ISAAC) was 44.1% in probiotic group and 61.8% in placebo group (p=0.033). There was no other significant differences.

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Although the prevalence of asthma did not differ significantly in the whole cohort or in the aforementioned subgroups, wheezing attacks during the previous 12 months were reported by 8.5% of the probiotic group and 14.7% of the placebo group. A breakdown of the responses to asthma-related ISAAC questions is presented in Table 5. Wheezing attacks during the previous 12 months represented the only variable with a significant difference.

There was no significant difference in average SCORAD scores in the whole cohort (n=470, 1.94 in the probiotic group and 2.33 in the placebo group, p = 0.214) or in the caesarean delivered subgroup (n=79, 2.26 in the probiotic group and 2.17 in the placebo group, p = 0.701). In the whole cohort 13.5% of the participants in the probiotic group and 17.4% of the participants of the placebo group had SCORAD higher than 0 (p=0.253). In the caesaren delivered subgroup 13.5% of the probiotic group and 19.0% of the placebo group had SCORAD higher than 0 (p=0.508).

Growth was similar in the two groups (Appendices, Table 7). Essential haematological values were normal in the study population, and there were no statistically significant differences between the groups (Appendices, Table 8). There were no statistically significant differences in PEF measurements. The average PEF result was 356 L/min in the probiotic group (n=153) and 359 L/min (n=143) in the placebo group. For boys, the average PEF result was 360 L/min in the probiotic group and 370 L/min in the placebo group. For girls, the average PEF result was 351 L/min in the probiotic group and 348 L/min in the placebo group. The average best PEF result of three was 359 L/min in the probiotic group and 364 L/min in the placebo group.

**Discussion**

Early probiotic treatment did not show an overall preventive effect on doctor-diagnosed allergic diseases in 13-year follow-up. However, in the Caesarean-delivered subgroup a statistically significant effect was observed in the incidence of any allergy, and eczema, as reported in the ISAAC questionnaire. Analysis of the caesarean delivered subgroup was not preplanned in the original study protocol. It should also be noted, that in this study design the birth-mode is a post-randomisation variable. There was no significant difference in SCORAD scores between probiotic and placebo groups. An important motivation behind this follow-up study was to investigate the possible effect of probiotic treatment on airway allergies, which mostly become manifest in later childhood.

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The probiotic group reported clearly fewer wheezing attacks in the previous 12 months, but this was not reflected in the overall prevalence of asthma or allergic rhinitis, or in PEF measurements. Unlike it was done in the 2- and 5-year follow-up, this follow-up did not include clinical evaluation by pediatrician, but children met a study nurse. The limitations of questionnaire-reported assessment has to be taken into an account.

We have reported numerous comparisons with p-values. Holm method was applied to address this issue. However, it must be taken into an account, that many of our outcome variables are interconnected due to the way they are calculated and they have also a strong clinical interconnection. Consideration is needed, when evaluating this statistical analysis. To our knowledge, this is the longest follow-up period in a probiotic allergy prevention trial with sensitisation analysis. The size of our cohort is still good 13 years after the intervention.

Sensitisation analysis provided valuable information to accompany the questionnaire data. Increased sensitisation was observed in the probiotic group of the whole cohort, but not in the Caesarean-delivered subgroup. On the basis of the results of our study, the development of sensitisation and that of allergic disease was surprisingly divergent. Probiotic treatment increased sensitisation and showed some tendency to result in less allergic disease in the whole cohort – and significant reduction in allergic disease was observed in a Caesarean-delivered subgroup. In a study by Kalliomäki et al.(17) a reduction in atopic eczema persisted at 7-year follow-up, but sensitisation was not reduced. It may be speculated, as has been proposed by Dotterud et al. (13), that the protective probiotic effect is not related to sensitisation, but may be related to an anti-inflammatory mechanism. The development of sensitisation is also different between different strains. In one cohort where L. acidophilus (LAVRI-A1) was used, the probiotic group had higher sensitisation compared to placebo group at 1-year-of-age. There was no difference in 2.5 years or at 5 years, which implies that L. acidophilus seems to prepone the development of sensitisation (26–28). In our cohort, colonisation of probiotic strains was observed at 6 months, but not anymore at 2 years of age (20).

Beneficial findings were seen in the Caesarean-delivered subgroup, where the prevalence of eczema was 18.9% in the probiotic group and 37.5% in the placebo group. This is in line with previous results from our cohort. In the 5-year follow-up Caesarean-delivered children in the probiotic group had

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significantly fewer IgE-associated allergies and less IgE-associated eczema (21). The prevalence of allergic diseases was very high in this high risk cohort (55.2% vs. 59.0%), which makes it convenient for observing possible preventative effects. However, the prevalence of allergic diseases was similar compared with that in another Finnish long-term follow-up study of high-risk cohorts (29), where the overall prevalence of allergic disease was 56.4% in probiotic groups and 46.6% in placebo groups. In one Norwegian perinatal prevention trial involving use of a probiotic mixture containing *L. rhamnosus* GG, *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* Bb-12, an effect on atopic dermatitis was seen mainly in children with non-atopic parents but not in children with atopic parents (13).

In previous literature, no significant differences have been found in lung-function measures, asthma or rhinitis in probiotic prevention trials (17–19). In our study we found fewer wheezing attacks in the probiotic group during the previous 12 months, but no effect on asthma prevalence. The same applies in a systematic review, where no protective association was found between perinatal use of probiotics and doctor-diagnosed asthma or childhood wheezing (30).

The participation rate in the study (63.1%) was good considering the long follow-up period. At 5-year follow-up the participation rate was 87.5%. The demographic characteristics of the groups were similar. There was no difference in daily use of probiotics at 13 years, but weekly use was somewhat more common in the probiotic group. Most importantly, there was no difference in the use of (non-study) probiotics during the early years (0–2 years) or at 3–5 years. It is believed that the microbiota and the immune system are most amenable to intervention by probiotics in the first year of life. Probiotics are already widely consumed, and the results of the trial could have possibly been more pronounced, without non-study probiotic usage.

Probiotic products are commonly used, but regular daily use is rare in our population. In our study, probiotic treatment started before birth and continued for six months in infancy. Different timings and modes of administration (to breast-feeding mother or infant directly) have to be considered when further studying the matter. According to a review published in 2017, neither prenatal, postnatal nor pre- and postnatal treatment is consistently effective in different study designs (12). The strongest results, however, have been seen in studies with prenatal maternal administration and postnatal supplementation directly to the infant (11,20,31). In one prenatal study with Lactobacillus GG, no effect on eczema was found (32). The authors presented a strong argument

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that probiotic treatment limited to the prenatal period is not effective for preventing eczema, or at least that Lactobacillus GG is not suitable for the purpose. In one study carried out in Sweden the treatment period lasted from 4–13 months of age, targeting the weaning period (19). In that study no long-term effect on any diagnosed allergic disease, airway inflammation or IgE sensitisation was found.

The viability of the used probiotic product is important. In one treatment study stopped in the pilot phase, heat-inactivated LGG was associated with adverse gastrointestinal symptoms and diarrhoea. The viable LGG used by the comparison group in the same study showed a tendency to lead to a decrease in SCORAD scores (33). In our study and in the majority of all studies in this field a viable probiotic product has been used. In our cohort we also tested for the presence of the probiotic strains in the faeces of the participants at birth, and at three, six and 24 months of age (21). The prevalence of the probiotic bacteria was significantly higher in the probiotic group than in the placebo group at six months of age (20).

World Allergy Organization guidelines (2015) suggest probiotics for pregnant women, breastfeeding women and for infants, when the child has a high risk for allergy. The benefit is considered to come mainly from preventing eczema (34). Pre- and postnatal probiotic intervention appears to be the most effective, and it has also been proposed that the question of the diversity of microbiota is multigenerational in wider sense. Correct selection of the probiotic strain is also important, and there is evidence concerning different effectiveness of the various probiotic strains. In a study with two treatment arms *Lactobacillus rhamnosus* HN001 was associated with a protective effect against eczema, but no effect was found as regards *Bifidobacterium animalis* subsp. *lactis* HN019 (31).

In conclusion, probiotic treatment did not reduce the prevalence of allergic disease in the whole-cohort in our 13-year follow-up study in a high-risk population. However, in the Caesarean-delivered subgroup, probiotics had a protective effect against any allergic disease and eczema, based on ISAAC questionnaire responses. Caesarean-delivered children appear to benefit more from affected maturation of the microbiota. It is quite commonly assumed, that the passage through birth canal is important in constituting the neonatal microbiome with the vaginal and stool bacteria, and therefore explains the caesarean delivered children's higher risk for allergic diseases. This view has, though, been strongly criticised (35), and requires further investigation. Nevertheless, it is well

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known that vaginally and caesarean delivered children have different microbiota composition during infancy (36). It is intriguing that the effect of early intervention is so strong that it is still detectable at the age of 13 years. The prevalence of allergic disease was high, and the consumption of non-study probiotic products after the trial phase was common but irregular. Further investigation is needed to confirm the long-term effect of probiotic intervention in different populations and to identify the most effective probiotic strains, synbiotic combinations and timing of administration.

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**Tables**

Table 1. Characteristics of the study children

| | **Probiotic** | **Placebo** |
|---|---|---|
| | n = 330 | n = 312 |
| Female | 50.3% | 50.0% |
| Birth weight (g, SD) | 3588 (465) | 3587 (503) |
| Birth height (cm, SD) | 50.6 (2.03) | 50.5 (1.99) |
| Mother's age at labor (years) | 31.1 | 31.6 |
| Maternal atopy | 81.5% | 81.1% |
| Paternal atopy | 57.9% | 58.3% |
| Both parents atopic | 39.4% | 39.4% |
| Caesarean delivery | 16.1% | 17.9% |
| Exclusive breast feeding >3 mo | 53.0% | 48.1% |
| Exclusive breast feeding >5 mo | 5.2% | 2.6% |
| Breast-feeding duration ≥6 mo | 74.8% | 68.3% |
| Smoking at home (0–2 years) | 27.9% | 29.8% |
| Smoking at home (13 years) | 16.7% | 18.3% |
| Smoker (13 years) | 0.6% | 1.0% |
| Dog/cat at home (0–2 years) | 18.5% | 17.9% |
| Dog/cat at home (13 years) | 38.5% | 33.3% |
| University-educated mother | 33.3% | 37.8% |
| University-educated father | 35.5% | 37.2% |
| Antibiotics during intervention | 21.5% | 27.9% |

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Table 2. Use of non-study probiotics

| | **Probiotic** | **Placebo** | **p (Pearson)** | **p (Fisher)** |
|---|---|---|---|---|
| **Early age** | | | | |
| Continuous 0–2 years | 25.5% | 25.0% | .895 | .928 |
| Continuous 3–5 years | 29.1% | 22.4% | .054 | .059 |
| **Previous 3 years** | | | | |
| Used probiotics, any freq. | 77.4% | 76.0% | .674 | .709 |
| **At 13 years** | | | | |
| During antibiotic course | 17.3% | 16% | .672 | .751 |
| During diarrhoeal disease | 12.7% | 17.6% | .083 | .098 |
| At wintertime regularly | 2.4% | 2.2% | .880 | 1.00 |
| Daily | 9.7% | 6.7% | .172 | .197 |
| Weekly | **16.1%** | **10.6%** | **.041 *** | **.049 *** |
| Sometimes | 48.2% | 52.2% | .304 | .306 |

In this table n (probiotic) = 330, n (placebo) = 312

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Table 3. Allergic disease and sensitisation in the probiotic and placebo groups at 13 years

| | Probiotic | % | Placebo | % | OR | 95% CI | p (Pearson) | p (Fisher) |
|---|---|---|---|---|---|---|---|---|
| **Primary endpoints** | | | | | | | | |
| Allergic disease | 182/330 | 55.2 | 184/312 | 59.0 | 0.855 | 0.626 – 1.170 | .328 | .339 |
| Allergic disease, specific IgE >0.7 kU/L | 105/246 | 42.7 | 85/213 | 39.9 | 1.121 | 0.722 – 1.628 | .547 | .569 |
| **Secondary endpoints** | | | | | | | | |
| **Sensitisation** | | | | | | | | |
| Any specific IgE >0.7 kU/L | 147/246 | 59.8 | 108/213 | 50.7 | 1.444 | 0.997 – 2.090 | .052 | .060 |
| Food-specific IgE >0.7 kU/L | 53/246 | 21.5 | 41/213 | 19.2 | 1.152 | 0.730 – 1.819 | .543 | .564 |
| Inhalant-specific IgE >0.7 kU/L | **146/246** | **59.3** | **106/213** | **49.8** | **1.474** | **1.018 – 2.134** | **.040 *** | **.048 *** |
| Any specific IgE >0.35 kU/L | 158/246 | 64.2 | 124/213 | 58.2 | 1.289 | 0.884 – 1.879 | .187 | .211 |
| Food-specific IgE >0.35 kU/L | 76/246 | 30.9 | 58/213 | 27.2 | 1.195 | 0.797 – 1.792 | .389 | .411 |
| Inhalant-specific IgE >0.35 kU/L | 153/246 | 62.2 | 119/213 | 55.9 | 1.300 | 0.894 – 1.888 | .169 | .183 |
| **Allergic disease (doctor-diagnosed, ever)** | | | | | | | | |
| Eczema all | 105/330 | 31.8 | 111/312 | 35.6 | 0.845 | 0.609 – 1.173 | .314 | .317 |
| Eczema, IgE-associated * | 61/246 | 24.8 | 55/213 | 25.8 | 0.947 | 0.621 – 1.444 | .801 | .830 |
| Asthma, all | 42/330 | 12.7 | 53/312 | 17.0 | 0.713 | 0.460 – 1.105 | .129 | .148 |
| Asthma, IgE-associated * | 25/246 | 10.2 | 26/213 | 12.2 | 0.814 | 0.454 – 1.457 | .487 | .552 |
| Rhinitis, IgE-associated † | 76/246 | 30.9 | 59/213 | 27.7 | 1.167 | 0.779 – 1.748 | .454 | .473 |
| Food allergy | 75/330 | 22.7 | 84/312 | 26.9 | 0.798 | 0.558 – 1.143 | .218 | .235 |
| Food allergy, IgE-associated ‡ | 25/246 | 10.2 | 26/213 | 12.2 | 0.814 | 0.454 – 1.457 | .487 | .552 |
| **Allergic disease (ISAAC, 12 months)** | | | | | | | | |
| Allergic disease | 158/330 | 47.9 | 161/312 | 51.6 | 0.862 | 0.632 – 1.174 | .346 | .385 |
| Allergic disease, specific IgE >0.7 kU/L | 103/246 | 41.9 | 80/213 | 37.6 | 1.197 | 0.823 – 1.743 | .347 | .390 |
| Eczema all | 77/330 | 23.3 | 78/312 | 25.0 | 0.913 | 0.636 – 1.311 | .622 | .645 |
| Eczema, IgE-associated * | 47/246 | 19.1 | 37/213 | 17.4 | 1.123 | 0.698 – 1.809 | .632 | .717 |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Asthma, all | 25/330 | 7.6 | 29/312 | 9.3 | 0.800 | 0.457 – 1.399 | .433 | .478 |
| Asthma, IgE-associated * | 19/246 | 7.7 | 13/213 | 6.1 | 1.288 | 0.620 – 2.674 | .497 | .583 |
| Rhinitis, IgE-associated † | 83/246 | 33.7 | 63/213 | 29.6 | 1.212 | .816 – 1.801 | .340 | .366 |

Values are presented as numbers and percentages of children. OR, unadjusted odds ratio. CI, confidence interval.

* Any specific IgE level >0.7 kU/L

† Any specific inhalant IgE level >0.7 kU/L

‡ Any specific food IgE level >0.7 kU/L

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Table 4. Allergic diseases and sensitisation in Caesarean-delivered children in the probiotic and placebo groups

| | Probiotic | % | Placebo | % | OR | 95% CI | p (Pearson) | p (Fisher) |
|---|---|---|---|---|---|---|---|---|
| **Primary endpoints** | | | | | | | | |
| Allergic disease | 34/53 | 64.2 | 37/56 | 66.1 | 0.919 | 0.418 – 2.021 | .833 | .844 |
| Allergic disease, specific IgE >0.7 kU/L | 15/38 | 39.5 | 20/40 | 50.0 | 0.652 | 0.266 – 1.602 | .350 | .372 |
| **Secondary endpoints** | | | | | | | | |
| **Sensitisation** | | | | | | | | |
| Any specific IgE >0.7 kU/L | 21/38 | 55.3 | 25/40 | 62.5 | 0.741 | 0.300 – 1.832 | .516 | .646 |
| Food-specific IgE >0.7 kU/L | 9/38 | 23.7 | 9/40 | 22.5 | 1.069 | 0.373 – 3.066 | .901 | 1.000 |
| Inhalant-specific IgE >0.7 kU/L | 20/38 | 52.6 | 25/40 | 62.5 | 0.667 | 0.270 – 1.645 | .378 | .492 |
| Any specific IgE >0.35 kU/L | 23/38 | 60.5 | 27/40 | 67.5 | 0.738 | 0.292 – 1.867 | .521 | .638 |
| Food-specific IgE >0.35 kU/L | 12/38 | 31.6 | 13/40 | 32.5 | 0.959 | 0.370 – 2.483 | .931 | 1.00 |
| Inhalant-specific IgE >0.35 kU/L | 22/38 | 57.9 | 26/40 | 65.0 | 0.740 | 0.297 – 1.848 | .519 | .642 |
| **Allergic disease (doctor-diagnosed, ever)** | | | | | | | | |
| Eczema all | 24/53 | 45.3 | 23/56 | 41.1 | 1.187 | 0.556 – 2.536 | .657 | .702 |
| Eczema, IgE-associated * | 11/38 | 28.9 | 12/40 | 30.0 | 0.951 | 0.359 – 2.518 | .919 | 1.000 |
| Asthma, all | 5/53 | 9.4 | 5/56 | 8.9 | 1.063 | 0.289 – 3.902 | .927 | 1.000 |
| Asthma, IgE-associated * | 3/38 | 7.9 | 2/40 | 5.0 | 1.629 | 0.257 – 10.328 | .602 | .671 |
| Rhinitis, IgE-associated † | 8/38 | 21.1 | 14/40 | 35.0 | 0.495 | 0.179 – 1.367 | .171 | .212 |
| Food allergy | 14/53 | 26.4 | 18/56 | 32.1 | 0.758 | 0.331 – 1.736 | .512 | .535 |
| Food allergy, IgE-associated ‡ | 3/38 | 7.9 | 6/40 | 15.0 | 0.486 | 0.112 – 2.100 | .326 | .482 |
| **Allergic disease (ISAAC, 12 months)** | | | | | | | | |
| Allergic disease | **22/53** | **41.5** | **38/56** | **67.9** | **0.336** | **.154 – 0.736** | **.006 *** | **.007 *** |
| Allergic disease, specific IgE >0.7 kU/L | 14/38 | 36.8 | 22/40 | 55.0 | 0.477 | 0.193 – 1.182 | .108 | .119 |
| Eczema all | **10/53** | **18.9** | **21/56** | **37.5** | **0.388** | **0.162 – 0.930** | **.031 *** | **.036 *** |
| Eczema, IgE-associated * | 6/38 | 15.8 | 12/40 | 30.0 | 0.438 | 0.145 – 1.319 | .137 | .181 |
| Asthma, all | 2/53 | 3.8 | 3/56 | 5.4 | 0.693 | 0.111 – 4.319 | .693 | 1.000 |
| Asthma, IgE-associated * | 2/38 | 5.3 | 1/40 | 2.5 | 2.167 | 0.188 – 24.929 | .526 | .610 |
| Rhinitis, IgE-associated † | 10/38 | 26.3 | 15/40 | 37.5 | 0.595 | 0.227 – 1.562 | .290 | .338 |

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Values are presented as numbers and percentages of children. OR, unadjusted odds ratio. CI, confidence interval. Analysing the caesarean delivered subgroup was not part of the original study protocol.

* Any specific IgE level >0.7 kU/L

† Any specific inhalant IgE level >0.7 kU/L

‡ Any specific food IgE level >0.7 kU/L

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Table 5. Breakdown of asthma-related ISAAC questions

| | **Probiotic** | **%** | **Placebo** | **%** | **OR (95% CI)** | **p (Pearson)** | **p (Fisher)** |
|---|---|---|---|---|---|---|---|
| Wheezing ever | 116/330 | 35.2 | 122/312 | 39.1 | 0.844 (0.613 – 1.163) | .300 | .327 |
| Wheezing 12 months | 49/330 | 14.8 | 56/312 | 17.9 | 0.797 (0.524 – 1.212) | .288 | .337 |
| Wheezing attacks 12months | **28/330** | **8.5** | **46/312** | **14.7** | **0.536 (0.326 – 0.882)** | **.013 *** | **.014 *** |
| Night wheezing 12 months | 9/330 | 2.7 | 15/312 | 4.8 | 0.555 (0.239 – 1.288) | .165 | .212 |
| Limited speaking 12 months | 7/330 | 2.1 | 12/312 | 3.8 | 0.542 (0.211 – 1.394) | .197 | .246 |
| Asthma ever | 51/330 | 15.5 | 57/312 | 18.3 | 0.818 (0.540 – 1.237) | .341 | .345 |
| Exercise wheezing 12 months | 42/330 | 12.7 | 44/312 | 14.1 | 0.888 (0.564 – 1.399) | .609 | .644 |
| Night cough 12 months | 46/330 | 13.9 | 60/312 | 19.2 | 0.680 (0.447 – 1.035) | .071 | .089 |

In this table n (probiotic) = 330, n (placebo) = 31

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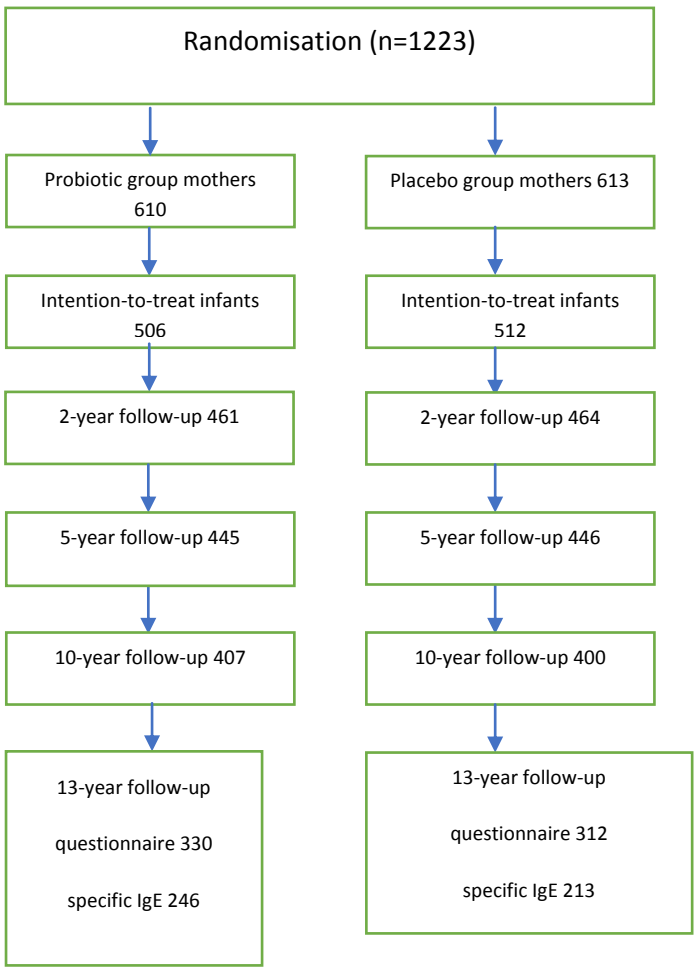
Chart 1. Flow chart of the study design

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**Appendices**

Table 6. Characteristics of the study children in different subgroups

| | **Dropout group*** | **Whole cohort (13y)** | **Caesarean delivered (13y)** | **Blood sample group (13y)** |
|---|---|---|---|---|
| | n = 312 | n= 642 | n = 109 | n = 459 |
| Female | 51.6% | 50.2% | 50.5% | 51.0% |
| Birth weight (g, SD) | 3606 (482) | 3588 (484) | 3592 (551) | 3582 (478) |
| Birth height (cm, SD) | 50.6 (1.85) | 50.6 (2.01) | 50.3 (2.14) | 50.5 (1.99) |
| Mother's age at labor (years) | 30.5 | 31.3 | 32.6 | 31.5 |
| Maternal atopy | 80.1% | 81.3% | 76.1% | 81.7% |
| Paternal atopy | 59.3% | 58.1% | 57.8% | 57.3% |
| Both parents atopic | 39.4% | 39.4% | 33.9% | 39.0% |
| Caesarean delivery | 16.7% | 17.0% | - | 17.0% |
| Exclusive breast feeding >3 mo | - | 50.6% | 42.2% | 50.3% |
| Exclusive breast feeding >5 mo | - | 3.9% | 2.8% | 3.9% |
| Breast-feeding duration ≥6 mo | - | 71.7% | 63.3% | 71.7% |
| Smoking at home (0–2 years) | - | 28.8% | 31.2% | 28.8% |
| Smoking at home (13 years) | - | 17.4% | 21.1% | 16.3% |
| Smoker (13 years) | - | 0.8% | 1.8% | 0.4% |
| Dog/cat at home (0–2 years) | - | 18.2% | 15.6% | 17.9% |
| Dog/cat at home (13 years) | - | 36.0% | 39.4% | 32.9% |
| University-educated mother | 36.2% | 35.5% | 31.2% | 37.0% |
| University-educated father | 33.0% | 36.3% | 36.7% | 37.3% |
| Antibiotics during intervention | 26.3% | 24.6% | 28.4% | 23.1% |

* 64 infants from the dropout group had to be ignored due to missing data.

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Table 7. Growth at 13 years

| | Probiotic | SD | Placebo | SD |
|---|---|---|---|---|
| Height (cm) | 160.0 | 7.2 | 160.1 | 7.1 |
| Weight (kg) | 49.5 | 9.75 | 49.2 | 9.65 |

In this table n (probiotic) = 251, n (placebo) = 219

Table 8. Essential haematological values at 13 years

| | Probiotic | SD | Placebo | SD |
|---|---|---|---|---|
| Leucocyte count (E9/L) | 5.64 | 1.51 | 5.48 | 1.45 |
| RBC count (E12/L) | 4.75 | 0.30 | 4.74 | 0.27 |
| Haemoglobin (g/L) | 136.50 | 7.82 | 135.59 | 7.28 |
| Haematocrit (%) | 39.95 | 2.16 | 39.67 | 1.92 |
| MCV (fl) | 84.13 | 3.11 | 83.78 | 3.35 |
| RDW (%) | 13.30 | 0.71 | 13.30 | 0.62 |
| MCH (pg) | 28.83 | 1.19 | 28.67 | 1.28 |
| MCHC (g/L) | 342.20 | 8.39 | 342.33 | 8.98 |
| Platelet count (E9/L) | 260.96 | 52.23 | 266.92 | 49.80 |

In this table n (probiotic) = 246, n (placebo) = 210

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**References**


1. Campbell DE, Mehr S. Fifty years of allergy: 1965-2015. *J Paediatr Child Health* 2015;**51**:91–93.
2. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;**347**:869–877.
3. Waser M, Michels KB, Bieli C, Floistrup H, Pershagen G, von Mutius E et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2007;**37**:661–670.
4. Chu S, Chen Q, Chen Y, Bao Y, Wu M, Zhang J. Cesarean section without medical indication and risk of childhood asthma, and attenuation by breastfeeding. *PloS One* 2017;**12**:e0184920.
5. Huang L, Chen Q, Zhao Y, Wang W, Fang F, Bao Y. Is elective cesarean section associated with a higher risk of asthma? A meta-analysis. *J Asthma Off J Assoc Care Asthma* 2015;**52**:16–25.
6. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med* 2016;**22**:1187–1191.
7. Ruokolainen L, Paalanen L, Karkman A, Laatikainen T, von Hertzen L, Vlasoff T et al. Significant disparities in allergy prevalence and microbiota between the young people in Finnish and Russian Karelia. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2017;**47**:665–674.
8. Sjogren YM, Jenmalm MC, Bottcher MF, Bjorksten B, Sverremark-Ekstrom E. Altered early infant gut microbiota in children developing allergy up to 5 years of age. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2009;**39**:518–526.
9. Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 1999;**29**:342–346.
10. Bertelsen RJ, Brantsaeter AL, Magnus MC, Haugen M, Myhre R, Jacobsson B et al. Probiotic milk consumption in pregnancy and infancy and subsequent childhood allergic diseases. *J Allergy Clin Immunol* 2014;**133**:165-71.e1-8.
11. 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 Lond Engl* 2001;**357**:1076–1079.
12. West CE, Dzidic M, Prescott SL, Jenmalm MC. Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention. *Allergol Int Off J Jpn Soc Allergol* 2017;**66**:529–538.
13. Dotterud CK, Storro O, Johnsen R, Oien T. Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial. *Br J Dermatol* 2010;**163**:616–623.


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14. Simpson MR, Dotterud CK, Storro O, Johnsen R, Oien T. Perinatal probiotic supplementation in the prevention of allergy related disease: 6 year follow up of a randomised controlled trial. *BMC Dermatol* 2015;**15**:13-015-0030–0031.

15. 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 J Br Soc Allergy Clin Immunol* 2013;**43**:1048–1057.

16. Gorissen DM, Rutten NB, Oostermeijer CM, Niers LE, Hoekstra MO, Rijkers GT et al. Preventive effects of selected probiotic strains on the development of asthma and allergic rhinitis in childhood. The Panda study. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2014;**44**:1431–1433.

17. 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**:1019–1021.

18. 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 Off Publ Eur Soc Pediatr Allergy Immunol* 2013;**24**:556–561.

19. West CE, Hammarstrom ML, Hernell O. Probiotics in primary prevention of allergic disease--follow-up at 8-9 years of age. *Allergy* 2013;**68**:1015–1020.

20. 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;**119**:192–198.

21. 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**:335–341.

22. 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 J Br Soc Allergy Clin Immunol* 2017;**47**:975–979.

23. Ellwood P, Asher MI, Beasley R, Clayton TO, Stewart AW, ISAAC Steering Committee. The international study of asthma and allergies in childhood (ISAAC): phase three rationale and methods. *Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis* 2005;**9**:10–16.

24. Pekkanen J, Remes ST, Husman T, Lindberg M, Kajosaari M, Koivikko A et al. Prevalence of asthma symptoms in video and written questionnaires among children in four regions of Finland. *Eur Respir J* 1997;**10**:1787–1794.

25. Ellwood P, Asher MI, Beasley R, Clayton TO, Stewart AW. *ISAAC Phase Three manual*. Auckland, New Zealand 2000

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Accepted Article

26. 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**:184–191.

27. Prescott SL, Wiltschut 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**:1481–1490.

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

29. Lundelin K, Poussa T, Salminen S, Isolauri E. Long-term safety and efficacy of perinatal probiotic intervention: Evidence from a follow-up study of four randomized, double-blind, placebo-controlled trials. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol* 2017;**28**:170–175.

30. Azad MB, Coneys JG, Kozyrskyj AL, Field CJ, Ramsey CD, Becker AB et al. Probiotic supplementation during pregnancy or infancy for the prevention of asthma and wheeze: systematic review and meta-analysis. *BMJ* 2013;**347**:f6471.

31. 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**:788–794.

32. Boyle RJ, Ismail IH, Kivivuori S, Licciardi PV, Robins-Browne RM, Mah L-J et al. Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial. *Allergy* 2011;**66**:509–516.

33. Kirjavainen PV, Salminen SJ, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. *J Pediatr Gastroenterol Nutr* 2003;**36**:223–227.

34. Fiocchi A, Pawankar R, Cuello-Garcia C, Ahn K, Al-Hammadi S, Agarwal A et al. World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. *World Allergy Organ J* 2015;**8**:4-015-0055-2. eCollection 2015.

35. Stinson LF, Payne MS, Keelan JA. A Critical Review of the Bacterial Baptism Hypothesis and the Impact of Cesarean Delivery on the Infant Microbiome. *Front Med* 2018;**5**:135.

36. Korpela K, de Vos WM. Early life colonization of the human gut: microbes matter everywhere. *Curr Opin Microbiol* 2018;**44**:70–78.

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