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Title

Postnatal probiotics and allergic disease in very preterm infants: sub-study to the *ProPrems* randomized trial

Short title

Probiotics, allergic disease and very preterm infants

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Abstract

Background: Probiotic supplementation to mothers and/or their term-born infants has been suggested to prevent allergic disease, in particular eczema; however, no studies have investigated probiotics for prevention of allergic diseases in very preterm infants. We evaluated the effect of a postnatal probiotic combination on development of allergic diseases in very preterm infants.

Methods: This sub-study was an *a priori* secondary outcome of the ProPrems multi-centre, double-blind, placebo-controlled randomized trial (ANZCTR:12607000144415). ProPrems randomized 1099 very preterm infants to receive a probiotic combination or placebo from soon after birth until discharge from hospital or term corrected age (CA), whichever was earlier. Allergic disease (eczema, atopic eczema, food allergy, wheeze, atopic sensitization) was assessed in a subgroup of ProPrems infants (n=281) as close to 12 months CA as possible by questionnaire, clinical examination and skin prick tests to common allergens.

Results: There was no difference in eczema incidence between the probiotic and placebo group (35[30%] of 118 infants vs 37[27%] of 137 infants, respectively, absolute difference 2.65%, 95% CI -8.45 to 13.75). Similarly, the incidence of atopic eczema (6[5%] of 118 vs 3[2%] of 137), food allergy (4[3%] of 124 vs 2[1%] of 154), wheeze (39[31%] of 127 vs 45[29%] of 154) and atopic sensitization (14[13%] of 106 vs 13[11%] of 123) were similar between the probiotic and placebo groups.

Conclusion: This study found no effect of postnatal administration of a probiotic combination on the incidence of allergic diseases or atopic sensitization in the first two years of life in children born very preterm. Evidence that probiotics are effective for prevention of allergic disease in premature infants remains lacking; adequately powered randomized controlled trials evaluating probiotic supplementation for allergy prevention in very preterm infants are needed.

Keywords

Atopic sensitization, Eczema, probiotics, preterm

Introduction

Allergic diseases cause significant disease burden worldwide and their prevalence has increased in recent times[1], in both high and low-resource countries[2]. Approaches to prevent eczema and other allergic diseases warrant investigation.

Alterations of the gut microbiota composition in early life, including reduced relative abundance of Proteobacteria, *Bifidobacterium, Akkermansia, Faecalibacterium* and *Lachnospira*[3-5], as well as altered colonisation patterns[6,7], have been associated with increased risk of allergic diseases such as eczema and atopic sensitization. Compared with term infants, preterm infants typically have a low diversity gut microbiota with low abundance of anaerobes, and higher abundance of potentially pathogenic organisms[8]. While alterations in gut microbiota composition or colonisation might be expected to result in increased risk of allergic diseases, studies have failed to consistently demonstrate a higher prevalence of atopy, eczema and food allergy in preterm compared with term infants[9-11]. However, preterm infants have been shown to have an increased risk of asthma and wheeze by meta-analysis compared to term infants, with very preterm infants at highest risk[12,13]. Preterm infants also have a high rate of hospital readmission with lower respiratory tract infections[14], and there is evidence to suggest that probiotic supplementation may reduce the risk of respiratory tract infections in term[15] and preterm infants[16].

Recent systematic reviews and meta-analyses have reported that probiotic supplementation to mothers during late pregnancy and to infants in the first 6-12 months of life is effective in reducing the frequency of eczema, atopic eczema and atopic sensitization in term-born children, particularly in infants with family history of atopy[17,18]. Postnatal only supplementation has failed to consistently demonstrate a benefit for prevention of allergic disease[19], however, few studies have been conducted exclusively in the postnatal period and results have been conflicting[20,21]. Furthermore, given probiotic effects are considered strain specific, pooling data of probiotic preparations that utilise different strains may be inappropriate[22]. Thus, caution should be exercised when interpreting the results of these meta-analyses.

Information on probiotic use for prevention of allergic disease in preterm infants is lacking. Recent results from a historically-controlled-cohort study showed no benefit of probiotic supplementation in preventing atopic dermatitis in infants born <30 weeks' gestation[23]. To date, there have been no randomized control trials (RCT) investigating the impact of probiotics on the primary prevention of allergic diseases and atopic sensitization in very preterm infants.

The primary objective of this study was to evaluate the effect of postnatal probiotic supplementation on the development of allergic diseases (eczema, atopic eczema, food allergy,

wheeze) and atopic sensitization, in very preterm infants in early childhood. The secondary objective of this study was to investigate the effect of probiotic supplementation on the number of infections during the first 2 years of life. This sub-study was an *a priori* secondary outcome of the ProPrems RCT[24,25].

Methods

ProPrems randomized controlled trial

ProPrems was a multicentre, double-blind, placebo controlled randomized trial designed to evaluate the effect of a probiotic combination administered daily to very preterm infants on the incidence of late-onset sepsis occurring before 40 weeks' corrected age (CA) or discharge home (ANZCTR# ACTRNO12607000144415)[24]. The study protocol and clinical outcomes have been published[24,25]. ProPrems was conducted in tertiary perinatal hospitals in Australia and New Zealand and approved by the Human Research Ethics Committees at each site and The Royal Children's Hospital where the allergy assessments were performed.

Briefly, 1,099 very preterm infants, born <32 weeks' gestation and weighing <1500g, were randomized 1:1 to receive a probiotic combination *Bifidobacterium infantis* (*BB-02;* 300 x 10^6), *Streptococcus thermophilus* (*TH-4;* 350 x 10^6) and *Bifidobacterium lactis* (*BB-12* 350 x 10^6) once daily (total of $1x10^9$ organisms per 1.5g in a maltodextrin base powder) or placebo (maltodextrin), from soon after birth (when infant was receiving at least 1mL of milk every 4 hours) until discharge from hospital or term CA, whichever occurred first. This was the only probiotic combination available at the time that had been previously evaluated in preterm infants[26]. Compliance to treatment was recorded as the number of days of study powder received.

ProPrems Allergy sub-study

Infants recruited at hospitals located in Victoria, Australia (n=683) were eligible to participate in this sub-study. Enrolled participants completed an allergy questionnaire administered by research staff. Participants were invited to attend an allergy follow-up visit at The Royal Children's Hospital, Victoria, Australia for clinical assessment and skin prick test (SPT) to common allergens. Infants were followed up as close to 12 months CA as possible. Due to limited resources, some infants were assessed up to 25 months CA. Parental informed consent for the allergy sub-study was obtained twice: initially during entry to the primary study and again at the time of the allergy follow-up assessment. Participants and assessors were blinded to treatment allocation at the time of follow-up.

Study Outcomes, allergy questionnaire and clinical measures

The primary outcomes of this sub-study were incidence of allergic diseases (eczema, atopic eczema, food allergy and wheeze) and atopic sensitization in the first two years of life. The secondary outcome of this sub-study was number of infections during the first 2 years of life.

The allergy questionnaire (Supplementary File 1) was adapted from the Probiotic Eczema Prevention Study[27] and collected demographic information, allergy outcomes and potential confounding factors. Socioeconomic status was established using the Index of Relative Socioeconomic Advantage and Disadvantage[28], and classified from Category 1-4, with Category 1 representing the most advantaged quartile.

Presence of eczema was defined as parent report of doctor diagnosis of eczema or fulfilment of the modified UK Working Party's Diagnostic Criteria for Atopic Dermatitis[29], i.e. a history of itchy skin in the preceding 12 months, plus ≥ 3 of the following: visible dermatitis involving the skin creases extensor surfaces and/or cheeks; a history of rash involving the skin creases, extensor surfaces and/or cheeks; a history of generally dry skin in the preceding 12 months; a history of asthma or allergic rhinitis (or history of atopic disease in a first degree relative). Atopic eczema was defined as eczema together with a positive SPT to one or more allergen. If eczema was reported in the questionnaire and was visible at the follow-up visit, the severity of the eczema was evaluated by the scoring atopic dermatitis index (SCORAD). If eczema was not present at the clinic visit, the SCORAD was not performed[30].

Food allergy was defined as parent report of doctor diagnosed cow milk, soy, egg, wheat or peanut allergy with or without a positive SPT to the relevant food allergen to capture both IgE and non-IgE mediated food allergy phenotypes. Wheeze was defined as any parental reported wheeze and number of infections during the first 2 years of life was defined as parent reported number of colds with fever lasting at least 3 days to ensure medically significant infections were captured.

Infants underwent SPT to a panel of six common allergens (egg white, cow's milk, peanut, cat hair, perennial rye grass pollen and house dust mite), a positive control (10mg histamine) and a negative control. The SPT was deemed valid if the diameter of the wheal for the positive control was ≥ 1mm than the negative control; a positive SPT was defined as a wheal diameter ≥ 3mm. Atopic sensitization was defined as a positive SPT to one or more allergen.

Statistical Analysis

The ProPrems RCT sample size was estimated on a baseline rate of 23% of at least one episode of

late-onset sepsis in infants born <32 weeks' gestation and <1500g. In this sub-study we recruited as many eligible participants as possible from the original study. No formal a priori power analysis was performed, as this sub-study was a secondary analysis of the original RCT. A retrospective power calculation was performed, which revealed that a sample size of n=255 provides 80% power to detect an absolute difference of 15% (30% vs 15%) between the placebo and probiotic groups with a 0.05 2-sideed significance level.

Perinatal characteristics, allergy risk factors and allergic disease outcomes were compared between probiotic and placebo treated infants. Proportions were calculated for categorical variables; continuous variables were summarized using medians and interquartile ranges (IQR). The absolute difference in disease outcomes between the probiotic and placebo groups was calculated. Logistic regression was used to examine the association between each allergic disease outcome and probiotic administration. Linear regression was used to investigate the association between number of infections and probiotic administration. Regression analyses were adjusted for baseline imbalances between the two groups. Chi-square test (or Fisher's exact where expected values <5) was used to compare differences in reactivity to specific SPT allergens between probiotic and placebo treated infants. Wilcoxon rank-sum test was used to compare differences in SCORAD scores between probiotic and placebo treated infants. Results were presented with 95% CI to indicate the degree of uncertainty around the estimates. Analyses were performed using Stata Version 14.0 (Stata Corp).

Results

Participant recruitment and characteristics

There were 683 infants recruited from Victorian hospitals in the ProPrems RCT. One-hundred and sixty-eight (25%) families declined participation in the allergy sub-study at the time of entry to the primary study, three families withdrew from the parent study and 59 infants were excluded (deceased at time of follow-up, did not receive allocated intervention, were not approached; Figure 1). Of the 453 families that were eligible to be approached to participate in the allergy sub-study, 165 (37%) either refused to participate or were uncontactable at the time of follow-up. Between December 2008 and March 2013, 288(64%) families consented to participate at the time of follow-up; seven families did not complete any study procedures. Data from 281 (62%) infants were analysed, 127 from the probiotic group and 154 from the placebo group.

All 281 participants completed the questionnaire, 46 (16%) by telephone. Two hundred and thirty-five infants (84%) underwent clinical examination and 229 (82%) underwent SPT. The age range of participants at allergy assessment was 8-25 months CA. The median CA at allergy assessment was

16 months CA (IQR=13-20) in the probiotic group and 16 months CA (IQR=14-19) in the placebo group. Demographic variables and possible risk factors for allergy were similar between the probiotic and placebo groups (Table 1), with the exception that a larger proportion of infants randomized to placebo were born before 28 weeks' gestation compared with those randomized to probiotic (47% vs 35%). However, the median gestational age of infants was similar between the two groups, 28.7 weeks (IQR=27-30.1) in the probiotic group and 28.1 weeks (IQR=26.6-29.9) in the placebo group.

Commencement of the parent study treatment intervention began at a median of four days of age (IQR=3-6) in both the probiotic and placebo group. The median number of treatment days was 65 (IQR=52-80).

The 281 infants included in this sub-study are representative of the Victorian ProPrems cohort with regard to demographic characteristics, as well as age at which study powder was commenced and duration of treatment (Supplementary table 1). A larger proportion of sub-study participants had a parent-reported family history of allergic disease compared to the Victorian ProPrems infants who did not participate in this allergy sub-study (71% vs 56%; Supplementary table 1).

Eczema

Presence of eczema could not be established in 26 infants due to missing data. Eczema outcome data was available for 255 of 281 (91%) infants, and atopic eczema outcome data was available for 255 of 281 (91%) infants. The overall incidence of eczema and atopic eczema in the first two years of life were 28% (95% CI 23 to 34%) and 4% (95% CI 2 to 7%), respectively. The incidence of eczema was similar in the probiotic and placebo group (35 [30%] of 118 infants vs 37 [27%] of 137 infants, respectively, absolute difference 2.65%, 95% CI -8.45 to 13.75, p=0.639). Similarly, the incidence of atopic eczema was similar in the probiotic and placebo group (6 [5%] of 118 vs 3 [2%] of 137, absolute difference 2.89%, 95% CI -1.76 to 7.56, p=0.212). There was no association between probiotic supplementation and eczema (AOR 1.12, 95% CI 0.64 to 1.93, Table 2) or atopic eczema (AOR 2.11, 95% CI 0.51 to 8.70) in unadjusted analyses or following adjustment for gestation <28 weeks.

SCORAD was performed in 25 infants. The median SCORAD in the probiotic supplemented infants was 11.5 (n=17, IQR 67.4-16.6) and it was marginally lower in the placebo infants (7.4 [n=9, IQR 3.9-8.6], p=0.200).

Atopic sensitization

Atopic sensitization outcomes were available for 229 of 281 (81%) infants. The incidence of atopic sensitization in the first two years of life was 12% (95% CI 8 to 17%). The incidence of atopic sensitization was similar in the probiotic and placebo group (14 [13%] of 106 vs 13 [11%] of 123, absolute difference 2.64%, 95% CI -5.79 to 11.07, p=0.537). There was no association between probiotic supplementation and atopic sensitization in unadjusted analyses or following adjustment for gestation <28 weeks (AOR 1.24, 95% CI 0.55 to 2.79; Table 2). Similarly, there was no difference in incidence of atopic sensitization to specific allergens between the probiotics and placebo groups (Supplementary table 2).

Food allergy and wheeze

Data were available from 278 of 281 (99%) infants for food allergy outcomes and from all 281 infants for wheeze outcomes. The incidence of doctor diagnosed food allergy in the first two years of life was 2% (95% CI 1 to 5%). Doctor diagnosis of food allergy was made in six infants. Two infants were allergic to cow's milk, two allergic to egg, one allergic to both cow's milk and egg, and one allergic to both egg and peanut. Four infants had an IgE mediated food allergy, confirmed by a positive SPT to the specific diagnosed food allergen/s. One infant with a diagnosis of cow's milk and egg allergy had a negative SPT to cow's milk but a positive SPT to egg, indicative of non-IgE mediated allergy to cow's milk. One infant with a diagnosis of cow's milk allergy had a negative SPT. The incidence of food allergy was similar in the probiotic and placebo group (4 [3%] of 124 vs 2 [1%] of 154, absolute difference 1.93%, 95% CI -1.66 to 5.51, p=0.272).

The incidence of parent reported wheeze in the first two years of life was 30% (95% CI 25 to 36%), and there was no difference between the probiotic and placebo infants (39 [31%] of 127 vs 45 [29%] of 154, absolute difference 1.49%, 95% CI -9.28 to 12.26, p=0.786).

There was no association between probiotic supplementation and significant difference in the incidence of food allergy (AOR 2.25, 95% CI 0.40 to 12.61) or wheeze (AOR 1.14, 95% CI 0.68 to 1.91) in unadjusted analyses or following adjustment for gestation <28 weeks.

Secondary outcome

Probiotic supplemented infants experienced fewer colds with fever lasting 3 or more days than placebo infants (adj. coefficient=-0.54[95% CI -1.06 to -0.01], p=0.044; adjusted for gestation <28 weeks).

Discussion

We found no significant effect of postnatal probiotic supplementation on the incidence of eczema, atopic eczema, food allergy, wheeze or atopic sensitization in the first two years of life in a subgroup of very preterm infants from the ProPrems RCT. However, as our study was powered to detect a 15% reduction in incidence, smaller magnitudes of effect cannot be excluded. Larger studies of probiotic supplementation for allergy prevention in preterm infants that are adequately powered to detect smaller effects are required.

The results of our study are consistent with a historically controlled cohort study that found no benefit of probiotic supplementation for prevention of atopic dermatitis in infants born <30 weeks' gestation[23]. Recent systematic reviews and meta-analyses suggest that probiotic supplementation in the prenatal and continuing into the postnatal period (to breastfeeding mother or infant) is important for prevention of allergic disease, particularly eczema[17,18]. Conversely, administration of probiotics exclusively during the postnatal period may not be effective in preventing allergic diseases in infants[18,19], with some studies finding a protective effect[31], while others report no effect after probiotic administration, and one reported increased rates of atopic and food sensitisation[21].

Varied incidences of atopic sensitization in preterm infants have been reported, with some studies linking preterm birth or low birth weight to an increased risk of atopy[32], and others showing the opposite[33] or finding no association[9]. Immune development during the antenatal and the immediate postnatal period of very preterm born infants differ markedly from those born at term, which can affect the development of tolerance versus sensitization to antigens[34].

We found the incidence of eczema in very preterm infants in the first two years of life to be 28% (95% CI, 23,34%). This is higher than previous reports. Barbarot et al reported the prevalence of eczema to be 17.6% within the first two years of life and 21.5% within the first five years of life in two different cohorts of preterm infants born between 29-32 weeks' gestation (Epipage cohort of infants born in 1997 and LIFT cohort of infants born 2003-2005, respectively)[11]. Similarly, Damm et al reported the prevalence of eczema to be 20.9% in a cohort of probiotic treated infants (born 2010-2013 at <30 weeks' gestation) and 17.1% in a cohort of preterm infants not treated with probiotics born 2007-2010 at < 30 weeks' gestation[23]. Geographic location, increasing prevalence of allergic disease, greater awareness of allergic diseases among parents and healthcare workers, different study populations and different ages at follow up combined with the fluctuating course of the disease and use of different diagnostic criteria are possible factors to explain the variation in reported incidence of eczema between infants in our study and previous studies.

Martin et al, evaluated data from 4,485 infants in the HealthNuts study and found a population prevalence of eczema at 12 months of age of 20.3% and a cumulative prevalence of parent-reported eczema of 28%[35]. The HealthNuts study investigated term infants from the same geographical area, at the same age and around the same time (2008-2011) and using similar methods for identifying eczema as our study (clinical examination and parent-report). As the prevalence of parent-reported eczema from Martin et al[35] is consistent with our findings we hypothesise that the prevalence of eczema does not differ greatly between preterm and term infants in Victoria, Australia.

Interestingly, parent-reported family history of atopic disease was very high in our cohort. Almost three-quarters of infants had a history of eczema, asthma, allergic rhinitis or food allergy in a first degree relative. Family history of allergies was reported as 45.8% in the Epipage preterm cohort and 36.9% in the LIFT preterm cohort[11] and. Damm et al reported a family history of atopic disease in 16% of children[23]. The high rate of family history of atopic disease in our cohort could explain our relatively high incidence of eczema in comparison to other studies. Furthermore, there was a high proportion of caesarean section births in our study, a high rate of breastfeeding (with 95% of infants receiving any breastmilk and over 50% receiving 5 months or more of breastfeeding) and just over half of the participants received antibiotics in the 6 months prior to the allergy follow-up visit. These factors are known to influence the gastrointestinal microbiota, which may in turn influence the development of eczema and other allergic diseases[4,8].

Although there is mechanistic data to suggest probiotics may have a role in the treatment of food allergy[36], there is very little evidence that such an approach is effective for induction of tolerance. To date, there have been no published trials investigating the use of probiotics to prevent food allergy in preterm infants. Similarly, there are no studies investigating probiotics for the prevention of asthma or wheeze in preterm infants. Our finding of no positive effect of probiotics on the prevalence of wheeze is consistent with meta-analysis findings in term-born infants[37]. Preterm infants are generally considered to be at increased risk for asthma[13,38]. However, as recurrent wheeze is also a primary manifestation of chronic lung disease of prematurity (bronchopulmonary dysplasia, BPD)[11], it is unclear whether wheeze in preterm infants represents atopic disease or a manifestation of BPD.

Interestingly, despite observing no effect of probiotic supplementation on wheeze, we found that infants receiving probiotic were less likely to experience frequent colds with fever compared to placebo infants. This is consistent with a previous report of reduced incidence of viral respiratory tract infections (primarily rhinovirus) in preterm infants receiving a probiotic (*Lactobacillus rhamnosus GG*) or prebiotic compared to placebo[16].

Systematic reviews and meta-analyses demonstrate safety of probiotics in preterm infants[39], although cases of bacteraemia with the administered probiotic strains have been reported[40]. No safety concerns arose during the ProPrems trial, with similar rates of AE reported in both the intervention and placebo groups. Furthermore, Jacobs et al.[41] followed *ProPrems* infants at 2-5 years CA and found that the probiotic combination of *Bifidobacterium infantis BB-02, Streptococcus thermophilus Th-4* and *Bifidobacterium lactis BB-12* did not negatively impact neurodevelopment or behaviour.

The World Allergy Organisation-McMaster University Guidelines for Allergic Disease Prevention recommend probiotic use in pregnancy and the postnatal period for infants at increased risk of developing allergy[42]. The guidelines do not address the circumstance of prematurity, and the authors of these guidelines acknowledged that recommendations were based on limited poor quality evidence and did not provide guidance on which probiotic strains/species to use, making it difficult to implement these guidelines in clinical practice. As probiotic effects are dose, disease and strain-specific[43], the lack of beneficial effect in our study may be attributable to the probiotic combination used, and evaluation of alternate probiotic species or strains may be warranted. Further adequately powered studies in premature infants are required to determine if there might be protective effects against development of allergic disease provided by probiotic supplementation in this population.

Strengths of this study include that it was a planned secondary outcome of a double-blinded, multi-centre RCT and the use of well-validated diagnostic criteria for eczema. Moreover, this is the first prospective study to examine the role of probiotic supplementation for prevention of allergic disease in preterm infants. Finally, stool samples are available for Victorian ProPrems participants, which will enable correlation of allergy with gut microbiota.

This study has limitations including recruitment bias and small sample size. Despite 453 families initially agreeing to participate, only 281 infants completed study procedures. Though we found limited differences in demographic characteristics between sub-study participants and the wider Victorian ProPrems cohort, it should be noted that a higher proportion of participants in this allergy sub-study had a parental report of family history of allergic disease compared to the Victorian ProPrems infants that did not participate in the sub-study. It is likely that infants with signs or parental suspicion of allergy were more likely to participate, therefore the true incidence of allergic disease may be lower than reported. However most probiotic prevention studies have included infants with high risk of allergies[44]. Another limitation is that several of the outcomes analysed were based on parenteral report, which may be limited by recall bias or may be biased depending on the

personal and/or family history of allergic disease. We had highly trained nurses assessing the questionnaire responses and the definition of eczema was based on 'doctor diagnosis' which should minimize bias.

We found no effect of the probiotic combination *Bifidobacterium infantis BB-02, Streptococcus thermophilus Th-4* and *Bifidobacterium lactis BB-12* on the incidence of allergic disease (eczema, atopic eczema, food allergy and wheeze) or atopic sensitization in very preterm infants in the first two years of life. Although current guidelines recommend probiotics for the prevention of allergic diseases in infants at high-risk of developing allergy, our findings highlight that there is insufficient evidence in premature infants and further well powered studies in the preterm setting are required to determine if the recommendation is applicable to this population.

Table 1. Participant characteristics

	Placebo N=154	Probiotic N=127
Perinatal characteristics:		
Gestational age, wk, median (IQR)	28.1 (26.6-29.9)	28.7 (27-30.1)
Gestational age		
< 28 weeks	72 (47)	44 (35)
≥ 28 weeks	82 (53)	83 (65)
Birth weight, g, median (IQR)	1062 (825-1275)	1081 (885-1295)
Birth weight		
<1000g	70 (45)	54 (43)
≥1000g	84 (55)	73 (57)
Gender		
Male	81 (53)	68 (54)
Female	73 (47)	59 (46)
Delivery Mode		
Vaginal	33 (21)	37 (29)
Breech Vaginal	7 (5)	3 (2)
C-section (no labour)	72 (47)	55 (43)
C-section (in labour)	42 (27)	32 (25)
Age commenced study powder, d, median (IQR)	4 (3-6)	4 (3-6)
Age study powder started (in days)		
< 4 days	67 (44)	55 (43)
≥ 4 days	87 (56)	72 (57)
Age finished study powder, d, median (IQR)	71 (60-88)	70 (54-81)
Length of supplementation, d, median (IQR)	65 (56-83)	65 (50-77)

Allergy risk variables at follow-up assessment:		
Corrected age at follow-up assessment, m, median (IQR)	16 (14-19)	16 (13-20)
Socioeconomic status[†]		
Cat. 1	47 (33)	36 (31)
Cat. 2	29 (21)	27 (23)
Cat. 3	44 (31)	32 (28)
Cat. 4	21 (15)	21 (18)
Family History of allergic disease[‡]		
No	46 (31)	33 (27)
Yes	103 (69)	87 (73)
Breastfeeding duration		
Never breastfed	8 (5)	5 (4)
Breastfed (1-4 months)	50 (33)	48 (38)
Breastfed (5-11 months)	56 (37)	52 (41)
Breastfed (≥ 12 months)	39 (25)	21 (17)
Age solids introduced		
< 6 months	69 (46)	54 (43)
≥ 6 months	82 (54)	72 (57)
Maternal antibiotics[§]		
No	89 (70)	80 (73)
Yes	39 (30)	30 (27)
Antibiotics given to infant in the last 6 months		
No	60 (52)	45 (47)
Yes	56 (48)	51 (53)
Age formula started		
< 4 months	86 (63)	74 (64)
≥ 4 months	50 (37)	42 (36)
Age formula stopped		
≤ 12 months	56 (41)	46 (40)
> 12 months	80 (58)	70 (60)
Dog exposure (inside or outside the home)		
No	85 (55)	67 (53)
Yes	69 (45)	60 (47)
Cat exposure (inside or outside the home)		
No	112 (73)	92 (73)
Yes	41 (27)	34 (27)
Farm residence		
No	148 (97)	119 (96)
Yes	5 (3)	5 (4)
Number of children living at home		

Nil	49 (32)	44 (35)
1 other child	64 (42)	53 (42)
2 other children,	27 (18)	19 (15)
≥ 3 other children	14 (9)	9 (7)
Day-care attendance in first 12 months of life		
No	100 (65)	82 (66)
Yes	54 (35)	43 (34)
Tobacco smoke exposure per day		
Nil	123 (80)	98 (77)
1-10 cigarettes	14 (9)	16 (13)
11-20 cigarettes	11 (7)	8 (6)
≥ 21 per day	6 (4)	5 (4)

Data presented as n(%) unless otherwise specified. Data missing for some variables for up to 69 infants and percentages were calculated using available data.

† Socio-economic status defined as 'Index of Relative Socio-economic Advantage and Disadvantage' [24]according to infant suburb of residence with Cat. 1 representing the highest quartile, Cat. 2 representing the second highest quartile, Cat. 3 representing the second lowest quartile and Cat. 4 representing the lowest quartile, in Australia.

‡ Family history of allergic disease defined as parent-reported history of eczema, asthma, allergic rhinitis or food allergy in the mother and/or father and/or sibling. Family history data missing for 11 infants.

§ ≥ 1 course of any antibiotics while breastfeeding

Table 2 Allergic disease outcomes

Allergic disease outcome	**TOTAL**[†] n/N (%); [95% CI]	**Probiotic**[†] n/N (%); [95% CI]	**Placebo**[†] n/N (%); [95% CI]	**Absolute difference (95% CI)**	**Crude OR**[‡] **(95% CI)**	**AOR** [§] **(95% CI)**	***P* value**[§]
Eczema	72/255 (28); [23,34]	35/118 (30); [21,39]	37/137 (27); [20,35]	2.65 (-8.45 to 13.75)	1.14 (0.66 to 1.97)	1.12 (0.64 to 1.93)	0.695
Atopic eczema	9/255 (4); [2,7]	6/118 (5); [2,11]	3/137 (2); [0,6]	2.89 (-1.76 to 7.56)	2.39 (0.59 to 9.79)	2.11 (0.51 to 8.70)	0.303
Atopic sensitization	27/229 (12); [8,17]	14/106 (13); [7,21]	13/123 (11); [6,17]	2.64 (-5.79 to 11.07)	1.29 (0.58 to 2.88)	1.24 (0.55 to 2.79)	0.605
Food allergy	6/278 (2); [1,5]	4/124 (3); [1,8]	2/154 (1); [0,5]	1.93 (-1.66 to 5.51)	2.53 (0.46 to 14.07)	2.25 (0.40 to 12.61)	0.358
Wheeze	84/281 (30); [25,36]	39/127 (31); [23,40]	45/154 (29); [22,37]	1.49 (-9.28 to 12.26)	1.07 (0.64 to 1.79)	1.14 (0.68 to 1.91)	0.629

Abbreviations: OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

[†] Denominator changes with each outcome, due to missing data for some variables (for example, a history of doctor diagnosed eczema was not reported by two parents and UK working party criteria was missing for 30 infants, resulting in missing eczema outcome data for 26 infants) and not all infants participated in each component of the allergy sub-study (for example, 229 infants out of 281 had a valid skin prick test).

[‡] Logistic regression was used to determine differences in allergic disease outcomes between probiotic and placebo assigned infants.

[§] Logistic regression was used to determine differences in allergic disease outcomes between probiotic and placebo assigned infants, analyses adjusted for gestation (gestation was expressed as above or below 28 weeks' gestation as a binary variable)

Figures

Figure 1. Consort flow diagram of study participants

[a] Victorian participant refers to an infant recruited at hospitals located in Victoria, Australia.

[b] Infant's parents not approached (n=7)

Supplementary Information

Supplementary file 1. Study questionnaire

Supplementary table 1. Comparison of demographics of sub-study participants and the wider Victorian ProPrems cohort

Supplementary table 2. Effect of probiotic supplementation on skin prick test reactivity

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