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Early gut colonisation by *Bifidobacterium breve* and *B. catenulatum* differentially modulates eczema risk in children at high-risk of developing allergic disease

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Running title: Early gut bifidobacterial colonisation and infant eczema

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ABSTRACT PAGE

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Early gut colonisation by *Bifidobacterium breve* and *B. catenulatum* differentially modulates eczema risk in children at high-risk of developing allergic disease

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Abstract

Background: An altered compositional signature and reduced diversity of early gut microbiota are linked to development of allergic disease. We investigated the relationship between dominant *Bifidobacterium* species during early postnatal period and subsequent development of allergic disease in the first year of life.

Methods: Faecal samples were collected at age 1 week, 1 month and 3 months from 117 infants at high risk of allergic disease. *Bifidobacterium* species were analysed by quantitative PCR and terminal restriction fragment length polymorphism. Infants were examined at 3, 6 and 12 months, and skin prick test performed at 12 months. Eczema was diagnosed according to the UK-Working Party criteria.

Results: The presence of *B. catenulatum* at 3 months was associated with a higher risk of developing eczema (OR_{adj}=4.5; 95% CI 1.56 to 13.05, p_{adj}=0.005). Infants colonised with *B. breve* at 1 week (OR_{adj}=0.29; 95% CI 0.09 to 0.95, p_{adj}=0.04) and 3 months (OR_{adj}=0.15; 95% CI 0.05 to 0.44, p_{adj}=0.00001) had a reduced risk of developing eczema. Furthermore, the presence of *B. breve* at 3 months was associated with a lower risk of atopic sensitisation at 12 months (OR_{adj}=0.38; 95% CI 0.15 to 0.98, p_{adj}=0.05). *B. breve* colonisation patterns were influenced by maternal allergic status, household pets and number of siblings.

Conclusions: Temporal variations in *Bifidobacterium* colonisation patterns early in life are associated with later development of eczema and/or atopic sensitisation in infants at high risk of allergic disease. Modulation of the early microbiota may provide a means to prevent eczema in high risk infants.

Key words

Atopic sensitisation; *Bifidobacterium breve*; *Bifidobacterium catenulatum*; eczema; gut microbiota; terminal restriction fragment length polymorphism

INTRODUCTION

The development of the intestinal microbiota occurs during the first few years of life, a window of time that corresponds to a critical stage of immune development and maturation (1). Colonisation with bifidobacteria may be particularly important as aberrancies in bifidobacterial number and composition have been identified prior to development of allergic disease (2, 3), suggesting a role in disease pathogenesis (4, 5). Reconstitution with *Bifidobacterium infantis* restores oral tolerance in germ free mice, but only if this occurs during the neonatal period (6), further supporting the role of bifidobacteria in immune development.

We and others have demonstrated that reduced microbial diversity early in life is associated with an increased risk of developing allergic disease in childhood (7-10). However, the specific species differences in the intestinal microbiota associated with allergy vary between different study cohorts and geographical regions (4, 11, 12) and no specific bifidobacterial species has been consistently identified as a key modifier of allergic disease risk. Nevertheless, the bifidobacterial fingerprint could be used as a potential biomarker to understand the intestinal status pointing to a putative dysbiosis; and conversely, the bifidobacteria composition could represent a target for prevention and/or treatment of allergic disease. A recent study demonstrated that mice with food allergy exhibit a specific gut microbiota signature (13), thus strengthening the putative role of microbe-host interactions in the development of allergic disease.

While microbial diversity is known to be important for immunological maturation, it is still debatable as to whether different species of microbiota within the same phylogroups have different impacts on allergic disease manifestations. In this study, we aimed to examine the relationship between the dominant *Bifidobacterium* species in the neonatal gut and the development of eczema or atopy during the first year of life.

METHODS

Study design

The infants included in this study were part of a randomised controlled trial (RCT) assessing the effectiveness of prenatally administered probiotics for the prevention of eczema in infants at high risk of developing allergic disease (Probiotic Eczema Prevention Study, registered with Cochrane Skin Group www.nottingham.ac.uk/ongoingskintrials/, trial no. 36). As described in detail elsewhere (14), 250 participating mothers were randomised to take 1.8 x 10^{10} colony-forming units *Lactobacillus rhamnosus* GG (LGG; Dicofarm SpA, Rome, Italy) or maltodextrin placebo once daily from 36 weeks' gestation until delivery. Infants were evaluated at 3, 6 and 12 months for the presence of eczema by an allergy nurse or paediatric allergist trained in eczema diagnosis and severity assessment. A questionnaire was completed on each occasion. Infant faecal samples were collected on days 7, 28, and 90 of life. The study was approved by the Human Research Ethics Committees of the Royal Children's Hospital and Mercy Hospital for Women. Written informed consent was obtained from all participants. The first 117 infants whose mothers enrolled in the original study (59 probiotic, 57 placebo) with adequate faecal samples formed the study sample that we report here. The aim of this nested study was to compare the *Bifidobacterium* species in (i) infants who developed eczema during the first year of life and those without eczema; and (ii) sensitised and non-sensitised infants.

Definition of eczema, IgE-associated eczema and sensitisation

Eczema was defined according to the UK Eczema Working Party criteria (15), namely, a history of itchy skin, scratching or rubbing plus at least three of the following: history of generally dry skin; history of skin rash affecting the flexures, cheeks or outer surfaces of the limbs; visible dermatitis at any study visit affecting the flexures, cheeks or outer surfaces of the limbs. Skin prick testing (SPT) (positive control 10% histamine chloride; negative control glycerin-saline) was performed at age 12

months to house dust mite, cat, ryegrass pollen, cow's milk, egg and peanut (Stallergens, Antony, France). Atopic sensitisation was defined as a SPT wheal diameter ≥ 3mm greater than the negative control to any single allergen tested. IgE-associated eczema was defined as the presence of both positive SPT and eczema at any time during the first 12 months.

Collection of faecal specimens

Infant faecal samples were collected at home by parents into a sterile faecal specimen container and placed immediately in a -20°C freezer where samples were stored until delivery to the laboratory on an ice pack. Samples were then aliquoted and stored frozen at -80^{o}C until they were processed. Different storage conditions of faecal samples can influence gram negative bacteria populations but is unlikely to influence bifidobacteria.

Quantification of total genus *Bifidobacterium* by real-time PCR

Total levels of *Bifidobacterium* in stool samples were determined by quantitative real-time PCR as described previously (16). To obtain a standard curve for the *Bifidobacterium* quantification, *B. infantis* DSM 20088 (Deutsche Sammlung von Mikroorganismen und Zellkulturen DSMZ, Germany) was grown in reinforced clostridial medium anaerobically for 20 hours at 37°C. A portion of the actively growing culture was subjected to DNA extraction as described previously (17), and another portion was plated on reinforced clostridial medium. Plates were incubated anaerobically at 37°C for 3 days, and the colonies were counted. Serial dilutions of the DNA and the corresponding plate counts were used as standards in the real-time PCR. The change in total *Bifidobacterium* concentration over time was analysed by using a generalised estimating equation approach with a normal distribution and an identity link. Since the distribution of bacterial counts was positively skewed, data were $\log_{10}$-transformed before analysis.

Identification of *Bifidobacterium* species by terminal restriction fragment length polymorphism peaks

Bacterial DNA was extracted as described previously (17) and stored at –20°C until analysis. *Bifidobacterium* species composition of the faecal samples were analysed by terminal restriction fragment length polymorphism (T-RFLP) as described elsewhere (18). Briefly, genus-specific primers were used to amplify a *Bifidobacterium* 16S rDNA fragment which was isolated by gel electrophoresis and extracted with QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). Purified DNA was subjected to enzymatic restriction by incubating 60 ng DNA with 10 U of *Alu*I (New England Biolabs Inc, Ipswich, MA, USA) at 37^{o}C for 4 hours. Enzymes were inactivated at the manufacturer's specified temperature. DNA was precipitated overnight and pellets were washed and air-dried. The digested products were analysed on a model 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA), at the Australian Genome Research Facility, Melbourne, Australia. Profiles were generated by analysing fragments up to 530 nucleotides in length. Twelve different Bifidobacterium strains were used to obtain the reference peak values for *Bifidobacterium* species identification (18). The identity of the cultures of the reference *Bifidobacterium* strains was confirmed by species-specific PCR as described (16). Based on the reference peaks, the T-RFLP peaks derived from the stool samples were allocated into 6 groups: the *B. adolescentis* group (*B. adolescentis* and *B. dentium*), *B. angulatum*, *B. lactis*, *B. breve,* the *B. catenulatum* group (*B. catenulatum* and *B. pseudocatenulatum*), and the *B. longum* group (*B. bifidum, B. longum* biotype *infantis* and *B. longum* biotype *longum*). Peaks representing an area of <1% of the total area of peaks were excluded from the analysis.

Statistical analysis

The Chi-square or Fisher's exact test was performed to determine whether the presence of different *Bifidobacterium* species and overall bifidobacteria at three different time points differed between children developing and not developing allergy by 12 months. The effects of confounding factors such as gender, treatment allocation groups, mode of delivery, exclusive breastfeeding for 4 months,

number of siblings, antibiotics and yoghurt intake during pregnancy, antibiotic use in infants, and family history of allergic disease were assessed using multiple logistic regression analysis and results are expressed as odds ratio (OR) with 95% confidence interval (CI). Spearman's rank correlation coefficient was calculated to investigate whether the total level of bifidobacteria in faecal samples at different time points correlated with possible confounding factors that may influence the neonatal gut microbiota. *P* values < 0.05 were considered to be significant. All calculations were performed using STATA version 11 software (StataCorp LP).

RESULTS

Characteristics of participating infants

Of 117 infants, 41 (35%) developed eczema at any time during their first year of life and 76 (65%) remained healthy. Skin prick testing was performed in 102 of 117 infants. Of these 102 infants, 35 (34.3%) had at least one positive SPT against tested allergens and therefore regarded as sensitised (atopic), while 67 infants (65.7%) were SPT negative (non-atopic). Of 41 infants who ever had eczema, 22 (53.7%) had IgE-associated eczema. In total, 33 infants (29.1%) were delivered via caesarean section and 83 (70.9%) were born vaginally. During the first 3 months when the faecal samples were collected, 45 infants (38.5%) were exclusively breastfed and 16 (13.7%) had received antibiotics. Table I shows the characteristics of 117 infants whose faecal samples were available for analysis. Among infants who developed eczema, 95% (39/41) had at least one parent affected with allergic disease and 41.5% (17/41) had both parents with allergic disease as compared to 88% (67/76) and 34.2% (26/76) respectively in those who remained healthy (p=0.2 and p=0.4, respectively). Infants attending day care were less likely to developed eczema (OR 0.44; 95% CI 0.20 to 0.98, p=0.04) when compared to those who did not attend day care. There were no significant associations between other influencing factors and eczema development.

Frequencies of Bifidobacterium species in children who developed allergic disease

The prevalence of different *Bifidobacterium* species in the gut microbiota of eczema and non-eczema infants at different time points is presented in Table II. Sample sizes vary as we were unable to analyse some bifidobacterial profiles because of unsuccessful restriction enzyme digestion or insufficient DNA for analysis.

At 1 week of age, almost three-quarters of infants were colonised with bifidobacteria (74.4%, 87/117), and almost all infants (92.3%, 108/117) had bifidobacteria by 3 months. There were no differences in the prevalence of overall bifidobacterial colonisation between eczema and non-eczema infants and between sensitised and non-sensitised infants at all time points evaluated. At age 1 week, 1 month and 3 months, the most frequently detected bifidobacterial species in both groups of infants belonged to the *B. longum* group.

Infants with eczema in the first 12 months were less often colonised with *B. breve* at 1 week and 3 months (p=0.05 and p=0.001, respectively) compared to those without eczema. There was also a trend for infants with eczema to be less often colonised with *B. breve* at 1 month (p=0.06) compared to infants with no eczema. In contrast, infants who ever had eczema were more often colonised with *B. catenulatum* at 1 and 3 months than healthy controls (p=0.05 and p=0.01, respectively), but not at 1 week. Figure 1 shows the prevalence of *B. breve* and *B. catenulatum* at the time points 1 week, 1 month and 3 months in the groups of infants who developed and did not develop eczema at 12 months.

With regards to sensitisation, we found that infants who were sensitised at 12 months were less often colonised with *B. breve* at 3 months as compared to those who were not sensitised (P=0.05), but there was no association between *B. breve* colonisation at 1 week (p=0.4) or 1 month (p=0.9) and sensitisation.

We also examined the relationship between presence of different *Bifidobacterium* species and development of IgE-associated eczema during the first 12 months. Infants with IgE-associated eczema were less often colonised with *B. breve* at 3 months compared to infants who did not develop any eczema (p=0.04), but not at 1 week or 1 month. *B. catenulatum* was detected more often at 1 and 3 months in infants with IgE-associated eczema than in those without eczema (p=0.03 and p=0.05 respectively), but not at 1 week.

The colonisation pattern of the other bifidobacteria (*B. adolescentis, B. angulatum, B. lactis* and *B. longum)* was similar in infants who did or did not develop eczema, IgE-associated eczema and atopic sensitisation.

Logistic regression modelling

Table III shows the association between each bifidobacterial species at age 1 week, 1 month and 3 months and the development of allergic manifestations (eczema, IgE-associated eczema and atopic sensitisation) within the first 12 months, tested using parametric tests and logistic regression with and without adjustment for treatment group allocation, gender, mode of delivery, and type of infant feeding, maternal yoghurt and antibiotic consumption during pregnancy, antibiotic use in infants, parental and sibling history of allergy, and number of siblings. The direction of the associations

specifically for *B. breve* and *B. catenulatum* was unaffected by the adjustment, although adjusted analyses enhanced the significance of the findings.

The adjusted odds ratio (ORadj) for eczema ever in infants colonised by *B. breve* was 0.29 (95% CI 0.09 to 0.95, p=0.04) if colonised at 1 week, 0.09 (95% CI 0.01 to 0.85, p=0.03) if colonised at 1 month, and 0.15 (95% CI 0.05 to 0.44, p=0.00001) if colonised at 3 months when compared to infants not colonised with *B. breve*. The risk of having eczema ever was significantly higher in infants colonised with *B. catenulatum* at 1 month (ORadj 5.26; CI 1.27 to 21.87, p=0.02) and 3 months (ORadj 4.51; 95% CI 1.56 to 13.05, p=0.005) compared with non-colonised infants (Table III).

Infants who were colonised with *B. breve* at 3 months were less likely to have IgE-associated eczema as compared to uncolonised infants (ORadj 0.29; CI 0.08 to 0.96, p=0.04). Infants colonised with *B. catenulatum* at 1 month (ORadj 9.04; CI 1.72 to 47.52. p=0.009) and 3 months (ORadj 4.11; CI 1.19 to 14.19, p=0.03) were at higher risk of developing IgE-associated eczema compared with infants not colonised with *B. catenulatum* (Table III).

Colonisation with *B. breve* at 3 months (but not at 1 week and 1 month) was protective against development of atopic sensitisation (ORadj 0.38; 95% CI 0.15 to 0.98, p=0.05), specifically against inhalant sensitisation (ORadj 0.10; 95% CI 0.01 to 0.85, p=0.03) but not against food sensitisation (p=0.1). However, colonisation with *B. catenulatum* was not associated with atopic sensitisation, food or inhalant sensitisation. (Table III).

Colonisation with other bifidobacteria, namely *B. adolescentis, B. angulatum, B. lactis* and *B. longum* at any time point assessed was not associated with any of the allergic disease outcomes (Table III).

External factors influence the early infant gut microbiota

We also analysed different external factors that could modify or modulate the composition of gut microbiota in early infancy. The number of family members correlated with total number of bifidobacteria at 1 month (*r*=0.3, p=0.03), and maternal antibiotic use in the last 4-6 weeks of pregnancy correlated with total number of bifidobacteria at 3 months (r=0.3, p=0.002).

In 1 week and 3 months faecal samples, total bifidobacteria levels were inversely correlated with mode of delivery (r=-0.4, p=0.0009; r=-0.2, p=0.04, respectively). For the allergic status of family members, total bifidobacteria at 1 week and 3 months were negatively correlated with maternal allergy and both parents with allergy (r=-0.3, p=0.01; r=-0.35, p=0.002, respectively). Similarly, the presence of bifidobacteria at 3 months was inversely correlated with sibling/s with eczema (r=-0.36, p=0.01). Exclusive breastfeeding for less than 4 months was negatively correlated with total number of bifidobacteria at 1 month (r=-0.3, p=0.05) and the starting of infant formula at age 3 months was negatively correlated with total number of bifidobacteria at 3 months (r=-0.4, p=0.03).

When specific bifidobacterial strains were examined, we found that infants who have allergic mothers were less likely to be colonised with *B. breve* at 1 week (OR 0.23; CI 0.08 to 0.61, p=0.003) and 3 months old (OR 0.29; CI 0.12 to 0.72, p=0.008). Likewise, infants who have both parents with allergic disease (OR 0.23; CI 0.07 to 0.72, p=0.01) and household pets (OR 0.28; CI 0.10 to 0.75,

p=0.01) were less likely to harbour *B. breve* at 1 week. However, infants with 2 or more siblings were more likely to be colonised with *B. breve* at 3 months old (OR 4.06; CI 1.41 to 11.70, p=0.009)

DISCUSSION

The presence of bifidobacteria has been associated with a lower risk of developing allergic disease (2, 3), although some studies have not shown any association (5, 19). In the present study, we assessed the association between different *Bifidobacterium* species and subsequent development of eczema and atopic sensitisation. We found that the presence of *B. breve* at age 1 week and 3 months was associated with a lower risk of developing eczema and sensitisation at age 12 months. In contrast, colonisation with *B. catenulatum* at age 3 months was associated with an increased risk of eczema development in the first year. Differences in the distribution of *Bifidobacterium* species have been observed between allergic and non-allergic infants in countries with high incidence of allergic diseases (4, 11, 12, 20, 21). Interestingly, the specific differences in patterns of colonisation with *Bifidobacterium* species differ in different geographical locations. Our finding that *B. catenulatum* was detected more often in infants who later developed allergic disease is consistent with data from New Zealand, the UK and Japan, and contrast with the study by Stsepetova et al in Estonia (21). We did not identify any association between *B. adolescentis* and allergic disease outcomes as Ouwehand did (20). Taken together, these findings suggest that differences in the levels of selected microbial species in allergic and healthy infants can be demonstrated consistently, but the bacterial species associated with either the presence or absence of atopic disease vary between different study cohorts and geographical regions. This highlights the importance of diversity in early life immune regulation and development of allergic disease as reduced diversity of early gut microbiota is associated with eczema development (7-10). Nevertheless, it remains likely that species of bacteria have specific immune effects that contribute to the functional signature of the microbiota. Disruption of the functional signature is likely to reflect combined effects of 1)

altered species composition and 2) reduced microbial diversity since increased diversity can allow for greater redundancy of functional activity. In the presence of reduced diversity the compositional signature becomes more important because there is reduced capacity for the less rich microbiota to compensate for reduced/absence of particular species. However, to assess functional signatures directly, it would be necessary to perform metabolomic studies, which were beyond the scope of this project.

Although we demonstrated that early *B. breve* and *B. catenulatum* colonisation can influence development of eczema and atopic sensitisation in infants, the mechanisms through which these bifidobacteria exert their effects are unknown. The immune effects of exposure to microorganisms are both species and strain dependent; for example, the capacity to induce FoxP3 Treg cells (22), and stimulate the production of different pro- and anti-inflammatory cytokines (22, 23). Hence it was not unexpected to identify differential effects associated with individual bifidobacterial species in our study. An infant-type *Bifidobacterium* such as *B. breve* induces the production of regulatory and Th1 cytokines. In a murine model of allergic inflammation, orally administered *B. breve* M-16V was shown to suppress the production of Th2 cytokine (IL-4) and IgE by inducing the secretion of regulatory (IL-10) and Th1 (IFN-γ) cytokines (24). Similarly, synbiotic (*B. breve* M-16V and prebiotics) treatment in asthmatic adults reduced allergen-induced Th2 (IL-4, IL-5 and IL-13) responses (25). *B. catenulatum* is generally categorised as an adult-type of *Bifidobacterium* species (26). In vitro studies demonstrated that *B. adolescentis* (another adult-type *Bifidobacterium*) isolated from allergic infants trigger the production of pro-inflammatory cytokines such as TNF-α, and IL-12 (27). *B. catenulatum* is also reported to be a strong inducer of IL-4 and IFN-γ production (28). Taken together, these study findings suggest that the development of infant eczema and/or atopy can be influenced by modulation of specific bifidobacteria patterns in early life, depending on their immunomodulatory properties, and this may be more apparent in the setting of reduced microbial diversity.

Two previous large scale studies failed to demonstrate any significant association between bifidobacterial colonisation and development of allergic/atopic outcomes in infancy (5, 19). Nonetheless, there are significant differences between their studies and ours, which might explain the discrepant findings. First, the study by Alderberth et al (19) defined 'atopic eczema' according to Williams' criteria and did not analyse IgE-associated eczema, whereas we used the UK working party criteria to define eczema. In the study by Penders et al (5), eczema was defined by parental reported symptoms with the UK working party criteria used only for those infants who were visited at home. Second, Alderberth et al (19) assessed only sensitisation to food, while we examined both food and inhalant sensitisation and indeed observed an association with inhalant but not food sensitisation. Third, Penders et al (5) evaluated only one faecal sample at one month of age, whilst we performed longitudinal analysis of three faecal samples collected at 1 week, 1 month and 3 months and identified greatest differences in early (1 week) and later (3 months) samples. However, the lack of significant findings for *B. breve* at 1 month could be due to lower statistical power to detect differences since the number of infants colonised with *B. breve* at 1 month were lower than those colonised at 1 week and 3 months of age.

Major strengths of our study are that we analysed all faecal samples prospectively (prior to the manifestation of atopic symptoms), in a blinded fashion, from a randomly selected subset of high risk infants, eliminating the chance of reverse causation. In addition, we performed logistic regression analyses to adjust for potential confounding factors, which have not been applied in previous studies. Few studies have prospectively explored the potential aetiological role of different bifidobacterial species in the development of allergic disease. Furthermore, some of the early studies pre-selected the study population according to allergic status – cases (allergic/atopic subjects) and controls (non-allergic/non-atopic/healthy subjects), potentially introducing bias from reverse causation (11).

One caveat is that at the time this work was conducted we have did not have access to species-specific primers for qPCR, and therefore were unable to quantify number of *B. breve* and *B. catenulatum* in the study samples. For this reason, we cannot make any conclusions about the sensitivity of our assay or the relative numbers of the different *Bifidobacterium* species. In this regard, it is worth noting that the resolution of T-RLFP is somewhat limited and not necessarily completely species-specific. Moreover, recent data indicate that the species *B. adolescentis* displays considerable genetic diversity and variable metabolic properties (29).

In this current study, the lifestyle factors that could influence microbial composition are also examined. Although early acquisition and higher numbers of bifidobacteria are seen in infants with older siblings (30), we are the first to demonstrate that infants with 2 or more siblings were more likely to harbour *B. breve* in the first 3 months of life. This supports the notion that siblings and family members may act as a reservoir to rapidly populate infants with beneficial commensal microbiota, and thus might protect infants against allergic disease. We also found that infants colonised with *B. breve* were more likely to have non-allergic mothers as well as non-allergic parents than infants harbouring few *B. breve*, suggesting that genetic propensity towards atopy and epigenetic factors may influence colonisation with certain *Bifidobacterium* species, which in turn may contribute to the risk of allergy. Mode of delivery, short duration of exclusive breastfeeding and age at introduction of infant formula were found to be inversely associated with early acquisition of bifidobacteria. Caesarean section and formula feeding are known to significantly influence the composition of infant microbiota by decreasing the numbers of bifidobacteria and increasing the colonisation of *Escherichia coli* and *Clostridium difficile* (30). Furthermore, compared with formula-fed infants, the gut microbiome of exclusively breastfed infants is dominant of protective gut bacteria such as bifidobacteria that utilise the complex sugars in human milk and interact more with host cells (31). The introduction of solid food profoundly impacts on the microbial ecology of

breastfed infants. Once dietary supplementation begins, microbiota profile of breast-fed-infants changes toward formula-fed-infants profile, with the significant increase in the count of Enterococci and Enterobacteria, and the appearance of *Bacteroides*, Clostridia, and other anaerobic Streptococci (31).

We previously reported no association between prenatal LGG supplementation and reduced risk of eczema in infants (14). This may be explained by the fact that prenatal LGG treatment did not modulate *B. breve* and *B. catenulatum* (18), which we have now identified as playing a role in modulating risk for development of eczema or atopy. Our current findings support the concept that factors which modulate the composition and diversity of endogenous intestinal microbiota, especially early in life, have the potential to modify the risk of allergic disease through modulation of the immune system. Interventions directed towards modifying microbial diversity and/or microbial composition in early life, such as probiotics, may offer an approach to reduce the risk for developing allergic disease. Animal and human studies have shown that administration of oral probiotics to pregnant mothers results in colonisation of the administered bacteria in the newborn offspring (32-34), and colonisation appears to be stable for at least 12 months (33). Furthermore, recent studies have demonstrated that several different *Bifidobacterium* species, particularly *B. breve*, are transmitted from mother to infant supporting the hypothesis that mother-to-infant transmission is occurring soon after birth (35, 36). As *B. breve* is known to be one of the predominant species in the infant's intestinal microbiota, it is tempting to suggest that administration of probiotics that promote colonisation with beneficial species such as *B. breve,* either directly (administration of *B. breve*) or indirectly (administration of other probiotic species that promote *B. breve* colonisation) to mothers before delivery might increase the transmission of this species to infants, hence providing a strategy for modulation of infant immune responses. This may in turn lead to clinical benefit such as a reduced risk of eczema development, although this was not demonstrated in our published RCT (14).

CONCLUSION

In this study we found significant associations between the proportions of specific bifidobacterial strains and subsequent allergy development – presence of *B. breve* was associated with reduced risk and *B. catenulatum* with higher risk of developing eczema. We also showed that bifidobacterial colonisation is related to both allergic heredity and IgE-mediated allergic disease at 12 months of age in a well-characterised cohort. These findings suggest that different species within *Bifidobacterium* may modulate immune responses in a different manner. The findings of reduced microbial diversity in allergic subjects possibly unmask the reduced capacity to compensate for functional activity of certain species. The differences in early gut microbiota composition that might exist between allergic and non-allergic subjects could be in part determined by genetic/epigenetic factors. Interventions that modulate infant gut microbiota such as prenatal and/or postnatal administration of probiotics may reduce the risk of eczema and one mechanism by which this is mediated may be by enhancing bifidobacterial diversity in the neonatal intestinal milieu early in life. Nevertheless, it should be noted that the finding of an association does not necessarily indicate causal relationship.

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Conflict of Interest: MLKT is a member of Medical Advisory Boards (Australia and New Zealand) for Nestle Nutrition Institute and Danone Nutricia, Global Scientific Advisory Board in Allergy and Immunity for Danone Nutricia; and has received speaker fees for symposia sponsored by Danone Nutricia. Other authors have not declared a conflict of interest with respect to this study.

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Table I. Characteristics of infants who developed eczema during the first 12 months of life

Variable	No eczema (n = 76)	Eczema (n = 41)	P value*
Sex (male), n (%)	44 (57.9%)	21 (51.2%)	0.6
Caesarean delivery, n (%)	19 (25%)	14 (34.1%)	0.2
Maternal allergy, n (%)	55 (72.4%)	35 (85.4%)	0.1
Paternal allergy, n (%)	38 (50%)	21 (51.2%)	0.8
Sibling/s with allergy, n (%)	37 (48.7%)	21 (51.2%)	0.3
Antibiotics during pregnancy, n (%)	4 (5.3%)	4 (9.8%)	0.4
Yoghurt during pregnancy (g/wk), median (IQR)	400 (100,700)	200 (0,900)	0.6#
Antibiotics in infants (first 3 months), n (%)	10 (13.2%)	6 (14.6%)	0.8
Number of children in family (≥ 2), n (%)	14 (18.4%)	6 (14.6%)	0.6
Household pets at 3 months, n (%)	39 (51.3%)	17 (41.5%)	0.3

Day care attendance, n (%)	55 (72.4%)	22 (53.7%)	0.04
Breastfed > 3 months, n (%)	62 (81.6%)	31 (75.6%)	0.6
At least 1 positive SPT (sensitised), n (%)	13 (17.1%)	22 (53.7%)	0.00001

*Pearson's chi-square test

#Mann-Whitney test

SPT, skin prick test; IQR, inter-quartile range

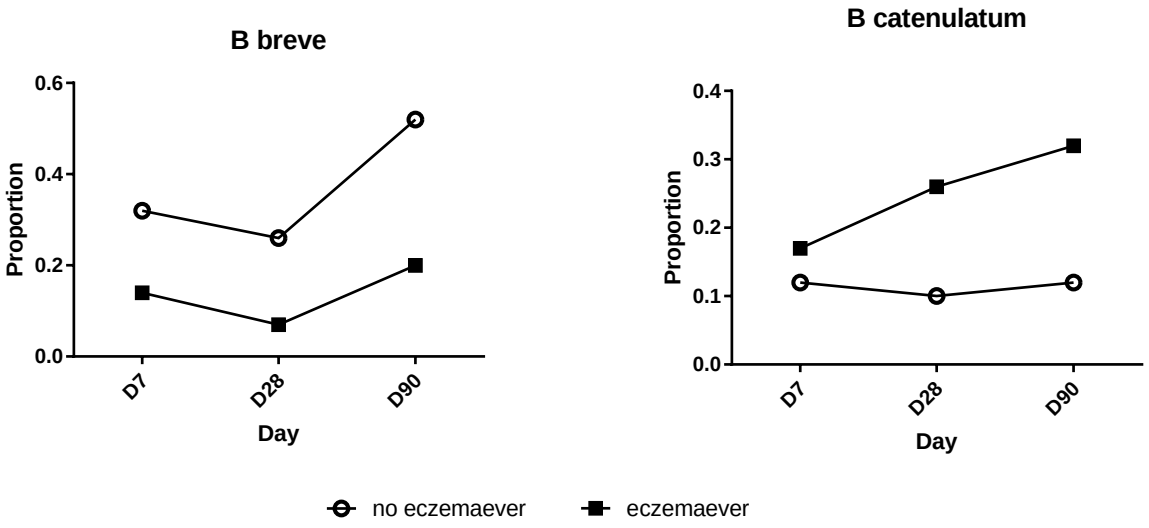

Figure 1. Prevalence of *Bifidobacterium breve* and *B. catenulatum* in the non-eczema and the eczema groups over time. The data presented is the proportion of *B. breve* and *B. catenulatum* colonisation at 1 week, 1 month and 3 months of age in infants who developed and did not develop eczema at the age of 12 months.

Table II. Proportion of infants colonised with bifidobacterial species during the first 3 months of life and allergy outcomes in the first 12 months / at 12 months

Bifidobacterial species detected in faeces §	No eczema ever	Eczema ever	No eczema at 12 month	Eczema at 12 month	No atopy	Atopy
Day 7						
Any, n (%)	58/65 (89.2)	29/35 (82.8)	62/68 (91.2)	17/22 (77.3)	51/57 (89.5)	26/31 (83.9)
B. adolescentis group, n (%)	14/65 (21.5)	6/35 (17.1)	14/68 (20.6)	5/22 (22.7)	12/57 (21)	6/31 (19.3)
B. angulatum, n (%)	9/65 (13.8)	9/35 (25.7)	10/68 (14.7)	6/22 (27.3)	10/57 (17.5)	5/31 (16.1)
B. breve, n (%)	21/65 (32.3)	5/35 (14.3)	19/68 (27.9)	5/22 (22.7)	14/57 (24.6)	10/31 (32.2)
B catenulatum group, n (%)	8/65 (12.3)	6/35 (17.1)	10/68 (14.7)	2/22 (9)	9/57 (15.8)	2/31 (6.4)
B. lactis	1/65 (1.54)	1/35 (2.86)	1/68 (1.47)	1/22 (4.5)	2/27 (7.4)	0
B. longum group, n (%)	33/65 (50.8)	18/35 (51.4)	39/68 (57.3)	8/22 (36.4)	32/57 (56.1)	13/31 (41.9)
Day 28						
Any, n (%)	40/42 (95.2)	19/22 (86.4)	42/45 (93.3)	11/13 (84.6)	34/37 (91.9)	18/20 (90)
B. adolescentis group, n (%)	11/50 (22)	5/26 (19.2)	11/52 (21.1)	4/17 (23.5)	9/46 (19.6)	6/22 (27.3)
B. angulatum, n (%)	9/50 (18)	4/26 (15.4)	10/52 (19.2)	3/17 (17.6)	10/46 (21.7)	3/22 (12.6)

B. breve, n (%)	13/50 (26)	2/26 (7.7)	12/52 (23.1)	1/17 (5.9)	9/46 (19.6)	4/22 (18.2)
B catenulatum group, n (%)	5/50 (10)	7/26 (26.9)	6/52 (11.5)	5/17 (29.4)	5/46 (10.9)	6/22 (27.3)
B. lactis	1/50 (2)	1/26 (3.8)	1/52 (1.9)	0	0	1/22 (4.5)
B. longum group, n (%)	34/50 (68)	16/26 (61.5)	49/71 (69)	30/40 (75)	33/46 (71.7)	12/22 (54.5)
Day 90						
Any¸ n (%)	69/71 (97.2)	39/40 (97.5)	71/73 (97.3)	24/25 (96)	58/61 (95.1)	35/35 (100)
B. adolescentis group, n (%)	21/71 (29.6)	10/40 (25)	19/73 (26)	8/25 (32)	14/61 (22.9)	13/35 (37.1)
B. angulatum, n (%)	13/71 (18.3)	7/40 (17.5)	12/73 (16.4)	6/25 (24)	11/61 (18)	7/35 (20)
B. breve, n (%)	37/71 (52.1)	8/40 (20)	34/73 (46.6)	6/25 (24)	30/61 (49.2)	10/35 (28.6)
B catenulatum group, n (%)	9/71 (12.7)	13/40 (32.5)	11/73 (15.1)	7/25 (28)	9/61 (14.7)	9/35 (25.7)
B. lactis	0	0	0	0	0	0
B. longum group, n (%)	49/71 (69)	30/40 (75)	52/73 (71.2)	18/25 (72)	41/61 (67.2)	27/35 (77.1)

§ numbers in table do not always add up to 117 due to missing bacterial count data or outcome data;

Table III. Association between bifidobacterial species colonisation and eczema and atopic sensitisation during the first 12 months of life

Bifidobacterial species detected in faeces	Eczema ever				Atopic sensitisation			
		P value				P value		
	OR adj (95% CI)	chi-square test	Logistic regression		OR adj (95% CI)	chi-square test	Logistic regression	
			Unadjusted	Adjusted#			Unadjusted	Adjusted#
Day 7								
Any	0.48 (0.13 to 1.80)	0.4	0.4	0.3	0.67 (0.17 to 2.62)	0.4	0.4	0.6
B. adolescentis group	0.57 (0.17 to 1.93)	0.6	0.6	0.4	0.68 (0.20 to 2.25)	0.8	0.8	0.5
B. angulatum	2.18 (0.70 to 6.76)	0.1	0.1	0.2	0.73 (0.20 to 2.62)	0.9	0.9	0.6
B. breve	0.29 (0.09 to 0.95)	0.05	0.06	**0.04***	1.58 (0.57 to 4.34)	0.4	0.4	0.4
B catenulatum group	2.3 (0.65 to 0.95)	0.5	0.5	0.2	0.36 (0.08 to 1.86)	0.2	0.2	0.2
B. lactis	0.91 (0.05 to 16.71)	0.6	0.6	0.9	-	-	-	-

B. longum group	1.31 (0.51 to 3.36)	0.9	0.9	0.6	0.63 (0.24 to 1.61)	0.2	0.2	0.3
Day 28								
Any	0.42 (0.05 to 3.22)	0.2	0.2	0.4	0.75 (0.10 to 5.72)	0.8	0.8	0.8
B. adolescentis group	0.93 (0.26 to 3.3)	0.8	0.8	0.9	1.49 (0.44 to 5.04)	0.5	0.5	0.5
B. angulatum	1.00 (0.26 to 3.93)	0.8	0.8	1.0	0.66 (0.15 to 2.85)	0.4	0.4	0.6
B. breve	0.09 (0.01 to 0.85)	0.06	0.07	**0.03***	0.94 (0.24 to 3.64)	0.9	0.9	0.9
B catenulatum group	5.26 (1.27 to 21.87)	0.05*	0.06	**0.02***	3.69 (0.93 to 14.67)	0.09	0.09	0.06
B. lactis	1.21 (0.06 to 24.47)	0.6	0.6	0.9	-	-	-	-
B. longum group	1.02 (0.33 to 3.13)	0.6	0.6	1.0	0.49 (0.16 to 1.51)	0.2	0.2	0.2
Day 90								
Any	0.90 (0.07 to 11.90)	0.9	0.9	0.9	-	-	-	-
B. adolescentis group	0.77 (0.30 to 1.99)	0.6	0.6	0.6	1.89 (0.74 to 4.85)	0.1	0.1	0.2
B. angulatum	1.04 (0.35 to 3.04)	0.9	0.9	0.9	1.19 (0.39 to 3.58)	0.8	0.8	0.6

B. breve	0.15 (0.05 to 0.44)	0.001*	0.001*	**0.00001***	0.38 (0.15 to 0.98)	0.05*	0.05*	0.05*
B catenulatum group	4.51 (1.56 to 13.05)	0.01*	0.01*	**0.005***	2.16 (0.74 to 6.29)	0.2	0.2	0.1
B. lactis	–	–	–	–	–	–	–	–
B. longum group	1.70 (0.64 to 4.52)	0.5	0.5	0.3	1.95 (0.70 to 5.41)	0.3	0.3	0.2

From logistic regression analysis, adjusted for treatment group allocation, sex, mode of delivery and type of infant feeding

*P value < 0.05