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Preventive effects of selected probiotic strains on the development of asthma and allergic rhinitis in childhood. The Panda-study.

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Evidence is mounting that progressive westernization and allergic disease are associated with disturbance of the intestinal microbial balance. This includes altered early colonization patterns and reduced bacterial diversity. In 2009 we reported results of early and long-term administration of

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selected probiotics to high-risk infants. All participating children had a positive family history of allergic disease, such as atopic eczema, food allergy, asthma or allergic rhinitis in either the mother, or the father plus an older sibling. In these children, administration of a probiotic mixture consisting of *Bifidobacterium bifidum*, *Bifidobacterium lactis* and *Lactococcus lactis* (Ecologic®Panda, Winclove Probiotics B.V., Amsterdam, The Netherlands) during pregnancy and during the first year of life was compared with placebo in a randomized controlled trial. Treatment with this combination of probiotics resulted in a preventive effect on the incidence of eczema, but not atopic eczema (eczema and IgE sensitization) (1). This preventive effect was established within the first 3 months of life, together with significant changes in the intestinal microbiota and decreased IL-5 production. No differences were observed in respiratory symptoms indicative for asthma or allergic rhinitis at the age of 2 years. In this study, we hypothesise a possible beneficial effect of long-term perinatal administration of selected probiotic strains on the prevalence of asthma, allergic rhinitis and eczema in these high risk children at the age of 6 years.

To that end, we approached all previous participants of the PandA study who had indicated that they were still willing to participate. The study was performed prospectively in a single-blinded (investigator blinded) design. At the child's age of 6 years, parents were asked to complete a slightly modified ISAAC questionnaire (2). The treating family doctor was asked for doctors diagnosed allergic diseases. Complete physical examination, blood sampling and lung function testing were performed. The study was approved by the Medical Ethics Committee of the University Medical Centre Utrecht, the Netherlands. Current asthma at age 6 years was defined as at least one of the following four criteria: doctors diagnosed asthma active in the past 12 months, parental reported wheezing in the past twelve months, use of asthma medication in the past twelve months and/or an at least 9 percent reversibility in the forced expiratory volume in half a second (FEV0.5) or in one second (FEV1) (3). Current allergic rhinitis was defined according to the ARIA guidelines (4). Food allergy was defined as doctor diagnosed food allergy, combined with sensitization to food allergens.

Eczema was defined according to the Williams UK Working Party's criteria. Sensitization was studied using measurement of specific IgE levels (IMMULITE 2000, Siemens Healthcare). Sensitization was defined as sIgE levels above 0.35 IU/ml.

From the initial 123 participants evaluated in the former PandA-study, 83 participants were willing to participate in the follow-up study at the age of 6 years. Baseline characteristics did not differ between the initial cohort and the patients who participated at follow-up. No differences were observed in basic descriptors between the placebo and intervention group. Parental smoking habits also were comparable. Mean age at follow-up was 5.9 years, 40 % were boys. The prevalence of allergic diseases in both groups is shown in Table 1. We observed a trend towards more allergic rhinitis in the intervention group. Additionally, there was no statistically significant difference in the severity of allergic rhinitis between the group receiving probiotics and the group receiving placebo. Furthermore, all spirometry measures were essentially equal in both groups. We also could not identify any statistically significant differences between exhaled bronchial NO nor nasal NO between both groups. Overall sensitization did not differ between the probiotics group and placebo group. Also, the total IgE levels did not differ between both groups.

Table 1. Outcomes of allergic disease at 6 years of age

	Probiotics (n=39)	Placebo (n=44)	Relative Risk (confidence interval)	p-value
Asthma	5	8	0.69 (0.24-1.93)	0.502
Allergic rhinitis	5	1	5.64 (0.68-46.2)	0.094
Food allergy	4	2	2.26 (0.44-11.6)	0.427
Eczema	13	14	1.05 (0.56-1.95)	0.883

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During this long term follow-up of a randomized placebo-controlled trial we could not demonstrate a beneficial effect on the development of allergic diseases at the age of 6 years from prenatal and one-year long postnatal use of a probiotic mixture consisting of *B. bifidum*, *B. lactis* and *Lc. lactis* (Ecologic®Panda). The reduction in asthma is not significant, nor is the increase in allergic rhinitis or food allergy. There is a trend towards a higher prevalence of allergic rhinitis in the intervention group. It has to be concluded that the positive effect on the prevalence of eczema, seen up to the age of 2 years, was not present anymore at the age of 6 years. No differences in number of sensitizations nor grade of sensitization were observed between both groups. Since the prevalence of allergic diseases, evaluated in our study, do correspond with the prevalence in high risk populations, we think our results do apply to this category.

Our study results, showing no preventive long-term effect of intervention with probiotics on allergic airway disease, are in line with other reports in literature (5). To date, only five studies have investigated the effect of probiotics on allergic airway diseases such as asthma and allergic rhinitis at the age above 4 years, which we think to be the earliest age to make a firm diagnosis of asthma. Kalliömaki *et al.* performed follow-up studies (6,7) when the children were 4 and 7 years old. They found that asthma and allergic rhinitis tended to be more common in the probiotic group and no differences in sensitization rates in both groups. We think our results for asthma are however more robust than theirs, since our diagnosis of asthma is based on lung function testing, whereas Kalliömaki based the diagnosis of asthma merely on (reimbursement of) prescription of asthma medication. Kuitunen *et al* (8) showed no effect of probiotics on asthma and allergic rhinitis at the age of 5 years in an elegant study with definitions of allergic disease comparable to ours in combining objective measurements with outcomes of questionnaires. They found an identical prevalence of allergic rhinitis in both groups at the same age as our population. As we did, they demonstrated a temporary effect of probiotics on the prevalence of eczema.

Abrahamson *et al.*, who used *L. reuteri*, found no reduction of asthma (RR 1.16 (0.33-4.10) (9), neither did Wickens *et al.* for *L. rhamnosus* HN001 (RR 0.95 (0.62-145)) (10) nor West *et al.* for *L. paracasei* spp *paracasei* F19 (RR 1.05 (0.39-2.81) (11).

Our study is in line with the other findings from literature. However, a relative strength of our study, as compared to above studies, is the selection method of the applied probiotics. Our study is the first in which probiotic bacteria were selected on the basis of *in vitro* modulation of cytokine production for use in an intervention study. *Bifidobacterium bifidum*, *B. lactis* and *Lc. lactis* were selected because of their good IL-10-inducing capacity *in vitro* as well as efficient inhibition of Th2-related cytokines IL-5 and IL-13.(12,13).

Starting supplementation of the probiotic intervention during pregnancy is another strength of our study, since prenatal start of the probiotics might be crucial to colonize mothers so that they transfer them to their offspring during vaginal delivery. An inevitable weakness of our study is the single-blind design of our randomised controlled trial at the stage of follow-up, because parents were already aware of which intervention their child received during the initial study. This might have resulted in parental bias. We believe this will not have strongly influenced our outcome since both the objective as well as the subjective (parental) parameters failed to show any effect of the intervention at this age. Furthermore, we had a loss to follow-up of 18% compared to the initially evaluated children, which might have induced some selection bias. However, the baseline characteristics of the initial group were identical to the baseline characteristics at the start of this follow up study, indicating that the loss to follow-up will not have significantly influenced our outcomes.

We conclude that a one-year long-term perinatal administration of a selected combination of probiotics (Ecologic® Panda) did demonstrate a beneficial effect on the development of eczema up to the age of two years. In accordance with other studies published thus far, to beneficial effect does not extend to the age of 6 years and does not lead to primary prevention of asthma. Given the fact that development of the gut microbiota composition may continue for at least the first 3 years of life

(14, and own, unpublished data), prolonged gut microbiota management may be required to achieve a long-lasting impact.

Conflict of interest

This study was funded by Agentschap NL, project FND 06-015. All authors have no conflicts of interest.

Reference list

1. Niers L, Martín R, Rijkers G, Sengers F, Timmerman H, van Uden N et al. The effects of selected probiotic strains on the development of eczema (the PandA study). *Allergy* 2009; **64**:1349-1358
2. Asher MI, Keil U, Anderson HR, Beasley R, Crane S, Martinez F et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;**8**:483–491.
3. Håland G, Carlsen KC, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M et al. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006; **355**:1682-1689
4. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update. *Allergy* 2008; **63**(Suppl86):8-160
5. Elazab N, Mendy A, Gasana J, Vieira ER, Quizon A, Forno E. Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. *Pediatrics* 2013 Sep; **132**:e666-76
6. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003;**361**:1869-1871

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7. 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
 8. 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
 9. Abrahamsson TR, Jakobsson T, Björkstén B, Oldaeus G, Jenmalm MC. No effect of probiotics on respiratory allergies: a seven-year follow-up of a randomized controlled trial in infancy. *Pediatr Allergy Immunol*. 2013;**24**:556-61
 10. Wickens K, Stanley TV, Mitchell EA, Barthow C, Fitzharris P, Purdie G et al. Early supplementation with *Lactobacillus rhamnosus* HN001 reduces eczema prevalence to 6 years: does it also reduce atopic sensitization? *Clin Exp Allergy*. 2013;**43**:1048-1057
 11. West CE, Hammarström ML, Hernell O. Probiotics in primary prevention of allergic disease - follow-up at 8-9 years of age. *Allergy*. 2013;**68**:1015-1020
 12. Niers LE, Timmerman HM, Rijkers GT, van Bleek GM, van Uden NO, Knol EF et al. Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin Exp Allergy* 2005;**35**:1481-1489
 13. Niers LE, Hoekstra MO, Timmerman HM, van Uden NO, de Graaf PM, Smits HH et al. Selection of probiotic bacteria for prevention of allergic diseases: immunomodulation of neonatal dendritic cells. *Clin Exp Immunol* 2007;**149**:344-352
 14. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;**486**:222-227