

Celiac disease by the age of 13 years is not associated with probiotics administration in infancy

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Abstract

Probiotics are theoretically promising in primary prevention of celiac disease (CD), but research evidence on the topic is scarce. We used the data and material of a clinical double-blind randomized placebo-controlled trial on primary allergy prevention (n=1223) to investigate in an exploratory study whether administration of a mix of pro- and prebiotics during late pregnancy and first 6 months of life was associated with prevalence of CD during 13-year follow-up. Children who fulfilled diagnostic criteria for CD (n=11) and subjects with a serum sample available for analyzing CD-antibodies (n=867) were included. CD or elevated tissue transglutaminase IgA antibodies were not associated with probiotics or placebo. Nor were there any associations with the mode of delivery, the duration of exclusive or total breastfeeding or respiratory infections during the first 2 years of life. Allergic diseases or sensitization by the age of 2 or 5 years were not clearly associated with the development of CD.

Keywords: clinical trial; allergy; prospective; primary prevention; early feeding

What is known

- Development of celiac disease (CD) requires genetic susceptibility and exposure to dietary gluten
- Early feeding does not appear to affect the risk of CD
- Gut microbiota in CD contains abnormally low quantities of *Lactobacilli* and *Bifidobacteria* strains

What is new

- Probiotic administration in late pregnancy and infancy did not affect the risk of CD in our trial
- Development of CD was not associated with allergy

Introduction

Celiac disease (CD) is a chronic immunological disorder elicited by dietary gluten intake in genetically susceptible individuals. The diagnosis in children is based either on the combination of high CD-specific antibodies, most importantly tissue transglutaminase IgA antibodies (tTGA,) and symptoms or on the combination of CD-specific antibodies and enteropathy observed in duodenal biopsies [1]. The disease risk is strongly associated with presence of HLA-DQ2.5 molecule encoded by *HLA-DQA1*05-DQB1*02 genes* and to a lesser extent with HLA-DQ8 encoded by *HLA-DQA1*03-DQB1*03:02 genes* [1]. Factors involved in the development of CD in addition to heredity, HLA haplotype and dietary gluten remain elusive. The timing of first gluten exposure and whether it is combined with breastfeeding or not, or the duration of breastfeeding do not appear to influence the risk of developing CD according to recent prospective studies [2,3]. CD is associated with various autoimmune diseases such as type 1 diabetes [4], and some evidence suggests an association also between CD and allergic diseases [5,6] implicating common mechanisms. Viral infections have been hypothesized to trigger CD, but scientific data to date is inconclusive [7,8]. Intestinal infections and the use of antibiotics in early life have been associated with increased risk for CD, which could be conveyed through altered gut microbiota [9]. The role of the gut microbiota in the development of CD is, however, unknown, as is the potential of probiotics in prevention [10]. We used the data and material of a clinical double-blind randomized placebo-controlled trial on primary allergy prevention [11,12] to investigate whether the incidence of CD in childhood was affected by the administration of a mix of pro- and prebiotics during late

pregnancy and first 6 months of life. We also analysed whether CD was associated with allergic diseases until the age of 5 years, or mode of delivery, early feeding, respiratory infections or the use of antibiotics in childhood.

Methods

Study population and protocol

The original clinical randomized double-blind placebo-controlled trial on primary allergy prevention with pro- and prebiotics comprised 1223 pregnant women who carried fetuses with a high risk for allergy (one or both parents had doctor-diagnosed allergic disease) [11,12]. The trial is registered in ClinicalTrials.gov with the registration number NCT00298337. Exclusion criteria were pre-maturity (<37 weeks of gestation), major malformations, or being a B-twin. The mothers took twice daily for 4 weeks before delivery one capsule containing a mixture of probiotics *Lactobacillus rhamnosus* GG (ATCC 53103) 5×10^9 colony-forming units (cfu), *L rhamnosus* LC705 (DSM 7061) 5×10^9 cfu, *Bifidobacterium breve* Bb99 (DSM 13692) 2×10^8 cfu, and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7076) 2×10^9 cfu, or placebo. Infants received from birth to the age of 6 months one opened capsule containing the same probiotics plus 0.8 g galacto-oligosaccharides in syrup once daily. Placebo capsules contained microcrystalline cellulose only. Placebo syrup contained no galacto-oligosaccharides. The active and placebo products were supplied by Valio (Helsinki, Finland). Parents were asked not to feed other pro- or prebiotics preparations during the intervention. The trial participants were invited for examination at the ages of 3 and 6 months and at 2 and 5 years, and additionally if parents reported that their child showed allergic

symptoms. Primary outcome measures were the cumulative incidence of any allergic disease until the age of 2 or 5 years. Sensitization was defined as one or more positive skin prick test results (≥ 3 mm larger than the negative control) and/or serum antigen-specific IgE > 0.35 kU/l to a wide panel of common allergens. Information on breastfeeding, infections, medications and any chronic diagnoses were collected with questionnaires at ages 1, 2, 3, 4 and 5 years. The latest follow-up on the trial population was at the age of approximately 13 years.

We checked in follow-up questionnaires and in patient records all subjects of the original trial for CD and other autoimmune disorders. We recorded individuals as having CD if they had received diagnosis based on CD-specific antibodies and duodenal pathology or if they had in the current analyses tTGA over ten times the normal limit, endomysium IgA antibodies (EMA) in dilution 1:50 or over and HLA-DQ2.5 or DQ8.

For this exploratory study, we included all subjects of the original trial that had confirmed CD by the end of year 2016 and subjects that we had a serum sample available for analyzing CD-antibodies at any of follow-up visits: at 2, 5 and 12 years. The study population thus comprised 878 children.

The ethics committee of the Children's Hospital of Helsinki University gave its approval for the study protocol. Mothers signed a written informed consent.

Laboratory methods

Sera were stored in -80°C . Tissue transglutaminase-2 IgA antibodies were measured with an enzyme-linked immunosorbent assay (ELISA) kit purchased from Eurospital, Italy (**Eu-tTG IgA New**). IgA-endomysium antibodies were analyzed in all specimens with

level of IgA-transglutaminase antibodies above the normal range (8 U) as described earlier [13]. Titer of 1:50 was considered as positive.

HLA genotyping for presence of DQB1*02, DQB1*03:02, DQA1*02:01 and DQA1*05 alleles was performed using lanthanide-labeled oligonucleotide probes specific for these alleles as described earlier [14].

Statistical methods

We categorized the study population into 3 groups: 1) CD, 2) possible CD: $8 < \text{tTGA} < 80$ and HLA-DQ 2.5 or DQ 8 or DQ 2.2, 3) CD excluded: $\text{tTGA} < 8$ or $8 < \text{tTGA} < 80$ and absence of HLA-DQ 2.5 or DQ 8 or DQ 2.2. We excluded from analyses children with $8 < \text{tTGA} < 80$ and no DNA sample available. We tested associations of these groups with pro- and prebiotics administration (vs. placebo), allergic diseases by the age of 2 years and 5 years and sensitization by the age of 5 years as well as type 1 diabetes by year 2016 (by at least the age of 13 years) with Pearson's Chi-squared test. We compared between these 3 groups with [analysis of variance \(ANOVA\)](#) tTGA levels, the duration of exclusive as well as total duration of breastfeeding, the number of respiratory infections and antibiotics regimens by the age of 2 years. We also compared with t-test these variables between subjects with CD and those with CD excluded. For final results, a p-value < 0.05 was considered significant. Analyses were performed with IBM SPSS Statistics for Windows (version 24.0, Armonk, NY, USA, IBM Corp.).

Results

Of the study population (n=878), 8 had received CD diagnosis by the end of year 2016, confirmed by duodenal pathology. The age at diagnosis ranged from 3.4 years to 13 years (mean 7.6. years). Antibody measurements found three subjects with tTGA over 10 times the detection limit combined with EMA over normal limit who were also HLA-DQ2.5 positive. They were thus concluded to have CD. Of these 11 subjects with CD, 10 had attended the follow-up at the age of 2 years and 9 at the age of 5 years.

DNA samples were available from 6 subjects with CD, and all of them had HLA-DQ2.5 haplotype and one of them also HLA-DQ8. Among children with $8 < \text{tTGA} < 80$ DNA sample was available in 51: 11 (22%) had HLA-DQ2.5 haplotype, 3 (6%) HLA-DQ2.2 and 11 (22%) HLA-DQ8. Seven children were excluded from the statistical analyses because they had $8 < \text{tTGA} < 80$ and no DNA sample was available and thus the possibility of CD could not be appraised. Among the 720 children with $\text{tTGA} < 8$ and DNA sample available, 135 (19%) had HLA-DQ2.5, 55 (8%) HLA DQ2.2 and 168 (23%) HLA-DQ8. We compared three groups: 1) CD (n=11), 2) possible CD: $8 < \text{tTGA} < 80$ and HLA-DQ 2.5 or DQ 8 or DQ 2.2 (n=22), 3) CD excluded: $\text{tTGA} < 8$ or $8 < \text{tTGA} < 80$ and absence of HLA-DQ 2.5 or DQ 8 or DQ 2.2 (n=838). We also compared the two extreme groups: CD and CD excluded. Comparisons between the three groups and between the two groups yielded similar results except for the association with allergic diseases and the use of antibiotics (Table 1). CD or possible CD were not associated with pro-and prebiotic treatment or placebo (Table 1). Nor was there any association with the mode of delivery, the duration of exclusive or total breastfeeding, the age of introduction of solid foods or

respiratory infections (Table 1). The use of antibiotics during the first 2 years of life was more common among children with CD than those with CD excluded (Table 1).

Allergic diseases by the age of 2 years or 5 years or sensitization by the age of 5 years were not associated with the development of CD when compared with the group of CD excluded, whereas between the three groups differences reached statistical significance in the prevalence of allergic diseases (Table 1). Type 1 diabetes was significantly associated with CD as well as possible CD (Table 1).

Discussion

Alterations in gut microbiota observed in CD patients [10, 15,16] and in infants with high genetic risk for CD [10, 17] suggest that CD prevention with probiotics is worth investigating. To our knowledge, however, no clinical trial has been reported to date on probiotic administration in early life for preventing CD. In our study population, CD developed as frequently in children who had received pro- and prebiotics during late pregnancy and first 6 months of life as in children who had received placebo. Although we used the data and material of a trial that was designed to study the prevention of allergy, the probiotic mix used in the trial contained probiotics relevant in CD, given that *Lactobacilli* strains and *Bifidobacteria* strains reportedly appear in abnormally low quantities in CD patients [15,16] and in infants with high genetic risk for CD [17]. We found no clear association between CD and allergic diseases. Unlike some previous studies reporting positive associations between atopy or asthma [5,6], in our study the diagnoses for allergic diseases were solidly based on clinical and laboratory examinations

and on uniform criteria. The numbers of subjects with CD and also of those with possible CD were, however, so small that further larger studies are needed to address this issue.

We also investigated whether factors that influence gut microbiota in early life differed between children with later CD and those without CD. The mode of delivery was not associated with later CD, which is in line with a prospective cohort study [18] but in contrast to a retrospective case-control study [19]. We also did not observe any association between the use of antibiotics in early childhood and later CD. In agreement with recent prospective studies [2,3], we observed no association between the duration of exclusive or any breastfeeding and the development of CD. Nor was there any association with the age at the introduction of solid foods, which according to the Finnish recommendations include cereals starting at the age of 5 months.

The strength of the study is that it is based on a randomized double-blind placebo-controlled trial that includes probiotics administration both in late pregnancy and first six months of life. Follow-up and diagnostics for allergic diseases were rigorous. Since the trial this exploratory study is based on was designed for investigating the prevention of allergic diseases, the size and familial risk profile of the study population are suboptimal for studying the risk of CD. For CD diagnoses we relied on patient record data and analyses on blood samples available. The prevalence of CD in the population was in line with that in general Finnish pediatric population [20]. Since all participants had family risk for allergy, the results may not, however, be directly applicable to general population. It is important to note that the probiotics administered in the trial were a mix

of four different strains. Any of the strains alone or in a different mix may convey different effects on microbiota.

Our negative result on the effect of early probiotics administration on the development of CD is not surprising given how complex issues the role of gut microbiome in CD and the potential of probiotics in primary prevention are. Further large, prospective studies are needed to decipher whether and how probiotics and which probiotics could prevent the development of CD.

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Table 1. Comparison between 3 groups (1) celiac disease (CD) (n=11), 2) possible CD: $8 < tTGA < 80$ and HLA-DQ 2.5 or DQ 8 or DQ 2.2 (n=25), 3) CD excluded: $tTGA < 8$ or $8 < tTGA < 80$ and absence of HLA-DQ 2.5 or DQ 8 or DQ 2.2) and 2 groups: CD and CD excluded

	Celiac disease n=11	Possible CD n=22	CD excluded n=838	p *Pearson's χ^2 , 3 groups † Pearson's χ^2 , 2 groups # ANOVA ¤ T test
pro- & prebiotics, n	6 (55%)	13 (59%)	413 (49%)	0.6* 0.8 †
cesarean section	1 (9%)	3 (14%)	148 (18%)	0.8* 0.7 †
exclusive breast feeding, months; mean (std;n)	3.3 (2.0;10)	2.6(1.9;21)	2.5 (1.9;806)	0.4 # 0.4 ¤
total breastfeeding, months; mean (std;n)	9.4 (5.9;9)	9.4 (5.0;19)	8.8 (5.3;716)	0.9 # 0.6 ¤
age at introduction of solid foods, months; mean (std;n)	4.1 (1.1;10)	4.1 (0.8;22)	4.0 (0.8;813)	0.6 # 0.6 ¤
respiratory infections by 2y, mean (std;n)	5.7 (5.8;11)	5.0 (3.3;22)	4.4 (3.4;838)	0.4 # 0.07 ¤
antibiotic treatments by 2y, mean (std;n)	3.2 (5.6;11)	2.0 (1.8;22)	2.1 (2.5;838)	0.4 # 0.000 ¤
tTGA at 2 y, mean (std; n)	153 (89;5)	6.8 (17;18)	1.6(11;621)	0.000 #
tTGA at 5 y, mean (std; n)	110 (84;5)	11.6 (38;19)	1.0 (1.4;708)	0.000 #

tTGA at 12 y, mean (std; n)	8.5 (5.4;3)	19 (11;21)	4.1 (4.6;432)	0.000 #
allergic disease by 2y, n/total n at 2y follow-up	1/10 (10%)	14/22 (64%)	253/821 (32%)	0.002 * 0.1 †
sensitization by 2y, n/total n at 2y follow-up	2/10 (20%)	10/22 (45%)	271/817 (33%)	0.3 * 0.4 †
allergic disease with sensitization by 2y, n/total n at 2y follow-up	1/10 (10%)	7/22 (32%)	133/818 (16%)	0.1* 0.6 †
allergic disease by 5y, n/total n at 5y follow-up	3/9 (33%)	17/22 (77%)	440/810 (54%)	0.04* 0.2†
sensitization by 5y, n/total n at 5y follow-up	4/9 (44%)	14/21 (67%)	385/802 (48%)	0.2* 0.8 †
allergic disease with sensitization by 5y, n/total n at 5y follow-up	2/9 (22%)	12/21 (57%)	290/802 (36%)	0.1* 0.4 †
Type 1 diabetes	1 (9%)	1 (5%)	8 (1%)	0.01* 0.01†