ORIGINAL ARTICLE

Randomized Feeding Intervention in Infants at High Risk for Celiac Disease

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ABSTRACT

BACKGROUND

A window of opportunity has been suggested for reducing the risk of celiac disease by introducing gluten to infants at 4 to 6 months of age.

METHODS

We performed a multicenter, randomized, double-blind, placebo-controlled dietary-intervention study involving 944 children who were positive for HLA-DQ2 or HLA-DQ8 and had at least one first-degree relative with celiac disease. From 16 to 24 weeks of age, 475 participants received 100 mg of immunologically active gluten daily, and 469 received placebo. Anti–transglutaminase type 2 and antigliadin antibodies were periodically measured. The primary outcome was the frequency of biopsy-confirmed celiac disease at 3 years of age.

RESULTS

Celiac disease was confirmed by means of biopsies in 77 children. To avoid underestimation of the frequency of celiac disease, 3 additional children who received a diagnosis of celiac disease according to the 2012 European Society for Pediatric Gastroenterology, Hepatology, and Nutrition diagnostic criteria (without having undergone biopsies) were included in the analyses (80 children; median age, 2.8 years; 59% were girls). The cumulative incidence of celiac disease among patients 3 years of age was 5.2% (95% confidence interval [CI], 3.6 to 6.8), with similar rates in the gluten group and the placebo group (5.9% [95% CI, 3.7 to 8.1] and 4.5% [95% CI, 2.5 to 6.5], respectively; hazard ratio in the gluten group, 1.23; 95% CI, 0.79 to 1.91). Rates of elevated levels of anti–transglutaminase type 2 and antigliadin antibodies were also similar in the two study groups (7.0% [95% CI, 4.7 to 9.4] in the gluten group and 5.7% [95% CI, 3.5 to 7.9] in the placebo group; hazard ratio, 1.14; 95% CI, 0.76 to 1.73). Breast-feeding, regardless of whether it was exclusive or whether it was ongoing during gluten introduction, did not significantly influence the development of celiac disease or the effect of the intervention.

CONCLUSIONS

As compared with placebo, the introduction of small quantities of gluten at 16 to 24 weeks of age did not reduce the risk of celiac disease by 3 years of age in this group of high-risk children. (Funded by the European Commission and others; PreventCD Current Controlled Trials number, ISRCTN74582487.)

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Celiac disease, an immune-mediated systemic disorder elicited by gluten in genetically susceptible persons, is characterized by anti–transglutaminase type 2 antibodies (TG2A) and enteropathy.[1] The prevalence of celiac disease is 1 to 3% in the general population and approximately 10% among first-degree family members of patients with celiac disease.[2-10] Celiac disease is treated with a gluten-free diet. More than 95% of patients have the HLA-DQ2 heterodimer, either in the *cis* or *trans* configuration. Most of the remaining patients have the HLA-DQ8 heterodimer or half of the HLA-DQ2 heterodimer (DQB1*02).[1,8,11-14] However, more than 25% of the general population has these haplotypes,[8,13] indicating that additional factors are involved in disease development.

Celiac disease increases the overall risk of death,[15] reduces quality of life,[16] and has extensive negative economic consequences.[17,18] The health and quality of life of patients improve with a gluten-free diet, but primary prevention would be more beneficial.[19,20] Results from observational studies indicate that the development of oral tolerance for gluten is initiated early in life and that the mode of introducing gluten to infants may influence the risk of celiac disease in predisposed persons.[21-25] The results of these studies suggest that there is a window of opportunity at 4 to 6 months of age, when the first exposure to gluten should occur in order to decrease the risk of celiac disease.[24,25] The results of studies evaluating breast-feeding and the risk of celiac disease are inconclusive, since most of these studies were retrospective and associated with parental recall bias, and none included randomization or specified the quantities of gluten consumed.[23-27] At present, the true influence of early feeding on the development of celiac disease remains controversial.

To investigate the possible primary prevention of celiac disease, the European multicenter project Prevent Coeliac Disease (PreventCD, www.preventcd.com) was initiated.[19] It was hypothesized that the frequency of celiac disease at 3 years of age could be reduced by exposing genetically predisposed infants to small quantities of gluten at 16 to 24 weeks of age, preferably while they were still being breast-fed.

METHODS

STUDY DESIGN AND PARTICIPANTS

We performed a prospective, randomized, double-blind, placebo-controlled, dietary-intervention study. The first child was included on May 26, 2007, and the follow-up for this analysis closed on September 10, 2013, when the youngest study participant turned 3 years of age; the oldest participants were up to 6 years of age. Infants 0 to 3 months of age were recruited consecutively through celiac-disease organizations in Croatia, Germany, Hungary, Israel, Italy, the Netherlands, Poland, and Spain. Infants were required to have the HLA-DQ2, HLA-DQ8, or HLA-DQB1*02 heterodimer (centrally typed) and to have at least one first-degree family member with celiac disease, as confirmed by means of small-bowel biopsies. We excluded premature infants and those with trisomy 21 or Turner's syndrome (see the Supplementary Appendix, available with the full text of this article at NEJM.org).

INTERVENTION

We randomly assigned participants to receive either 200 mg of vital wheat gluten mixed with 1.8 g of lactose (equivalent to 100 mg of immunologically active gluten) or placebo (2 g of lactose), given daily for 8 weeks starting at 16 weeks of age (see the Supplementary Appendix). Previous assessment of the vital wheat gluten by means of enzyme-linked immunosorbent assay and Western blot analysis had shown the presence of gluten proteins typically found in wheat gluten. Randomization, stratified according to participating country, was performed with the use of variable block sizes ranging from 4 to 8 and with SPSS software, version 18.0 (SPSS). The investigators and the parents of the participants were unaware of the intervention assignments.

Adherence to the study assignment was assessed by means of frequent interviews with the parents (Table S1 in the Supplementary Appendix). Participants were considered to have adhered to the intervention assignment if at least 75% of the study material (gluten or placebo) was ingested and no additional gluten was consumed. After the intervention, parents were advised to introduce gluten gradually, using regular products and standardized recommendations (see the Supplementary Appendix).

OUTCOMES

The primary outcome was the frequency of celiac disease at 3 years of age. The diagnosis of celiac disease was based on the histologic findings of small-bowel biopsies, according to the 1990 criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN).[28] Secondary end points were the occurrence of symptoms and the immune response to gluten as indicated by elevated serum antibodies associated with celiac disease (antigliadin antibodies and TG2A) (see the Supplementary Appendix).

FOLLOW-UP AND ASSESSMENT OF CELIAC DISEASE

We periodically monitored health status, anthropometric variables, and feeding habits (i.e., breast-feeding and formula feeding), and we quantified gluten consumption[29] using standardized questionnaires (Table S1 in the Supplementary Appendix). Measurements of serum antigliadin antibodies and TG2A were performed centrally at least seven times during the first 3 years of age and then annually thereafter. The parents of children with elevated celiac disease–associated antibodies or with symptoms suggesting celiac disease were offered small-bowel biopsies to confirm the diagnosis in their child (see the Supplementary Appendix). The biopsy specimens were histologically assessed at the study sites and were also reviewed by an author who is a pathologist.[30] The age of the patient when the diagnostic biopsies were performed was considered to be the age at the diagnosis of celiac disease.

STUDY OVERSIGHT

The study was approved by the medical ethics committee at each participating center and complied with Good Clinical Practice guidelines (see the Supplementary Appendix). The authors vouch for the veracity and completeness of the data and analyses reported and for the adherence of the study to the protocol, available at NEJM.org.

From 2007 to 2011, the study did not have commercial support. After 2011, Thermo Fisher Scientific performed antibody assessments without charge, and together with Eurospital and Fria Bröd, Thermo Fisher Scientific partly funded the project progress meetings. The funding organizations had no role in the conception, design, or conduct of the study, in the analysis or interpretation of the data, or in the writing of the manuscript or the decision to submit it for publication.

STATISTICAL ANALYSIS

To detect a 50% reduction in the development of celiac disease in the gluten group at 3 years of age (5%, vs. 10% with placebo) with a two-sided significance level of 5% and with 80% power, we calculated that 474 children would be required in each group.[19] All the data were entered into a Web-based data-management application with the use of a central structured-query-language server database (NEN 7510 certified). A statistical analysis plan was published online before the randomization codes were opened (http://preventcd.com/images/stories/Publications/PreventCD_SAP_1_0.pdf) (see the Supplementary Appendix).

For estimating the cumulative incidence of celiac disease, Kaplan–Meier curves were calculated, with time defined as the patient's age at the diagnosis of celiac disease or at the last assessment or withdrawal from the study (when data were censored). For comparison, a log-rank test (two-sided) was used, stratified according to participating country. The hazard ratio for celiac disease in the gluten group, as compared with the placebo group (with 95% confidence intervals), is provided, on the basis of a Cox proportional-hazards regression analysis. The primary analysis was performed according to the intention-to-treat principle. Differences in the cumulative incidence of celiac disease were assessed according to the baseline variables by means of Cox proportional-hazards regression (multivariate) analysis and according to the duration of breast-feeding, daily gluten intake, and occurrence of infection by means of a landmark analysis (see the Supplementary Appendix). Different intervention effects were assessed in subgroups by including an interaction term between intervention and subgroup in the Cox proportional-hazards regression analysis. Analyses were performed with the use of SPSS software, version 20.0 (IBM).

RESULTS

CHARACTERISTICS OF THE PARTICIPANTS

The parents of 1343 children provided written informed consent for the study. A total of 963 children were randomly assigned to receive gluten (483 participants) or placebo (480) (Fig. 1, and the Supplementary Appendix). After ran-

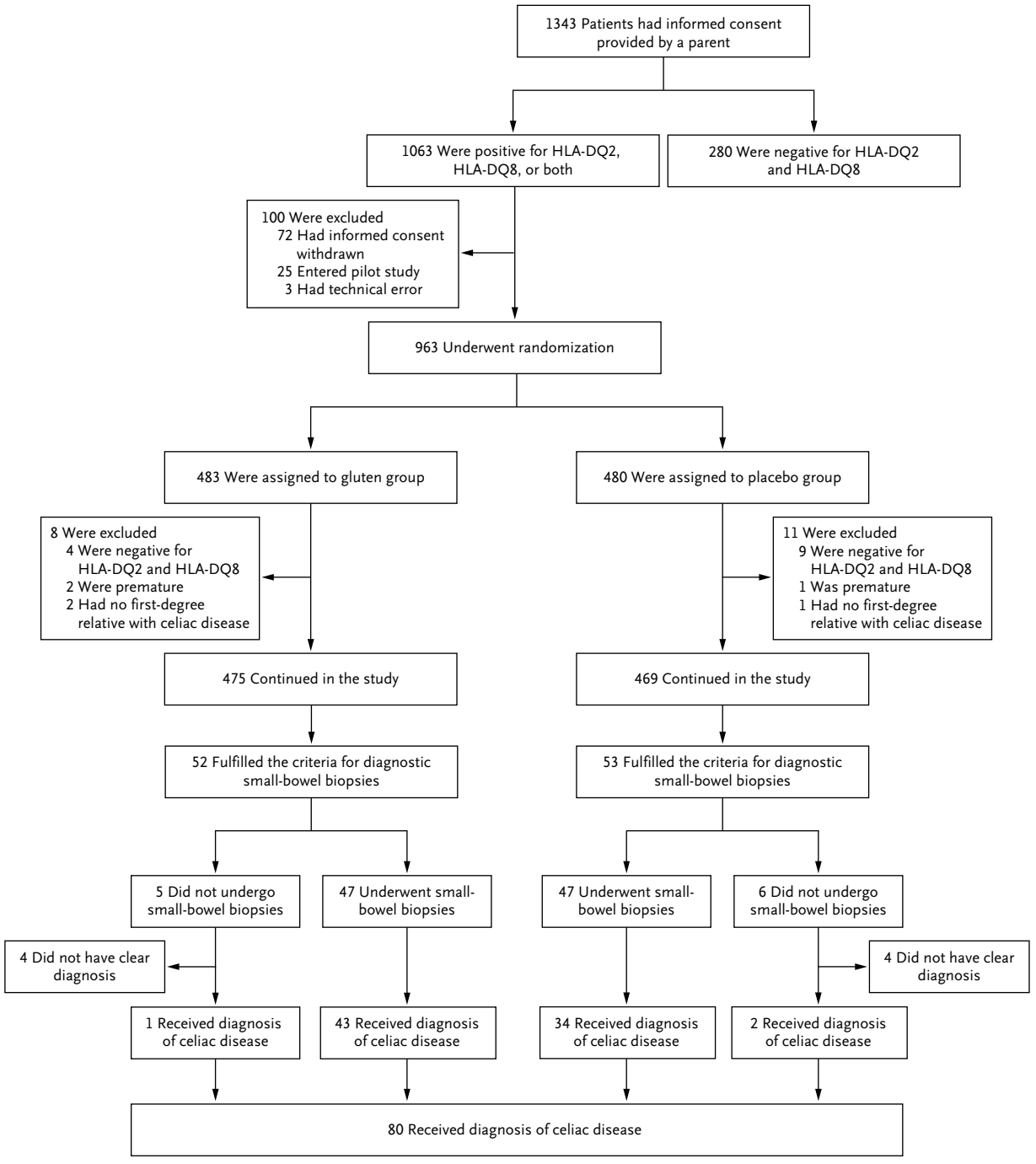


Figure 1. Randomization and Diagnosed Cases of Celiac Disease.

A total of 25 children were included in a pilot study to test the infrastructure of the study and were not included in the primary analysis. A total of 19 children underwent randomization in error and were excluded from the study. On the basis of histologic results of small-bowel biopsies, active celiac disease was ruled out in 17 children, although 3 of the 17 had potential celiac disease. There was no clear diagnosis in 8 asymptomatic children whose parents declined small-bowel biopsies on their behalf and who had transient levels of celiac disease–associated antibodies. Celiac disease was diagnosed in 3 children according to the 2012 European Society for Pediatric Gastroenterology, Hepatology, and Nutrition diagnostic criteria (without having undergone biopsies).[1]

domization, the number of children was reduced to 944 because 19 children did not fulfill the inclusion criteria. A total of 99 children (10.5%) did not adhere to the intervention assignment (59 children in the gluten group and 40 in the placebo group). A total of 141 children stopped participating before 3 years of age (withdrawal rate, 14.9%; 69 participants in the gluten group and 72 in the placebo group). A total of 59 children withdrew during the first year (6.2%), 49 during the second year (5.2%), and 33 during the third year (3.5%); the median follow-up was 4 years (range, 22 days to 6.3 years). The reasons for withdrawal were unknown for 57% of the children, were related to practical issues for 39% (e.g., blood sampling or travel distance to center), and were related to adverse events for 4% (see the Supplementary Appendix).

The baseline characteristics of the children were similarly distributed between the intervention groups, with the exception of homozygosity for HLA-DQ2 (Table 1). Data on breast-feeding were available for 943 children: 882 started breast-feeding; at 6 months of age, 527 (55.9%) were breast-fed, and 265 (28.1%) were breast-fed without complementary feeding except for the intervention product. Of the 455 mothers with celiac disease, 431 were consuming a gluten-free diet during pregnancy and lactation. Rotavirus vaccination was performed in 211 children (22.4%), either before the intervention (176 children) or during the intervention (35).

DIAGNOSIS OF CELIAC DISEASE

The numbers of children who met the criteria to undergo small-bowel biopsies are shown in Figure 1. A total of 101 small-bowel biopsies were performed in 94 children (Table 2, and the Supplementary Appendix). Celiac disease was confirmed by means of biopsies in 77 children. To avoid underestimation of the frequency of celiac disease, 3 additional children, whose parents declined biopsies on behalf of their children but who met the 2012 ESPGHAN diagnostic criteria,[1] were considered to have celiac disease in all analyses (Fig. 1).

The median age of the 80 children at diagnosis was 2.8 years (range, 1.1 to 5.6), and all the children had an elevated level of TG2A; 59% were girls. The most frequent symptoms were abdominal distention (in 20 children) and diarrhea (in 19). The cumulative incidence of celiac disease at 3, 4, and 5 years of age was 5.2% (95% confidence interval [CI], 3.6 to 6.8), 8.8% (95% CI, 6.6 to 11.0), and 12.1% (95% CI, 9.2 to 15.0), respectively (Table S2 and Fig. S1 in the Supplementary Appendix). Celiac disease was significantly more frequent in girls; at 3 years of age, the cumulative incidence among girls and boys was 7.2% and 3.4%, respectively; at 4 years of age, 11.8% and 6.1%; and at 5 years of age, 14.5% and 9.9% ($P=0.04$ by the log-rank test, $P=0.02$ by multivariate analysis) (Table S2 in the Supplementary Appendix). The disease developed significantly more frequently and earlier in the group of children who were homozygous for HLA-DQ2 (DR3–DQ2/DR3–DQ2 or DR3–DQ2/DR7–DQ2) than in the other HLA risk groups,[4] with cumulative incidences at 3, 4, and 5 years of age of 14.9%, 23.9%, and 26.9%, respectively ($P<0.001$) (Table S2 and Fig. S2 in the Supplementary Appendix).

Breast-feeding did not influence the development of celiac disease. The cumulative incidences at 3 years of age among children who were not breast-fed, were breast-fed for 3 or fewer months, were breast-fed for 4 or 5 months, or were breast-fed for 6 or more months were 7.3%, 4.4%, 8.2%, and 4.4%, respectively ($P=0.28$). Similar cumulative incidences at 3 years of age were observed among children who were never exclusively breast-fed or were breast-fed exclusively for 3 months or less, for 4 or 5 months, and for 6 months or more (5.0%, 9.1%, 5.3%, and 2.7%, respectively; $P=0.45$). Country of origin and the number and type of affected family members were also not related to the development of disease (Table S2 in the Supplementary Appendix), nor were rotavirus vaccination, gastrointestinal or respiratory tract infection, and mean daily gluten intake (see the Supplementary Appendix).

DEVELOPMENT OF CELIAC DISEASE IN RELATION TO THE INTERVENTION

The intervention with gluten, as compared with placebo, did not have a significant effect on the frequency of development of celiac disease, with cumulative incidences at 3 years of age of 5.9% (95% CI, 3.7 to 8.1) and 4.5% (95% CI, 2.5 to 6.5), respectively ($P=0.47$ by a stratified log-rank test; hazard ratio, 1.23; 95% CI, 0.79 to 1.91) (Fig. 2A). The duration of breast-feeding, whether exclusive or not, did not significantly influence the effect

Table 1. Characteristics of the Participating Children.*

Characteristic	Gluten (N=475)	Placebo (N=469)
Age at end of follow-up for this analysis — yr		
Mean	4.9	5.0
Range	3.1–6.5	3.1–6.6
Female sex — no. (%)	228 (48.0)	226 (48.2)
Gestational age — wk		
Mean	39.1	39.2
Range	34–43	35–42
Birth weight — g		
Mean	3316	3346
Range	1730–5000†	2000–4740
Country — no. (%)		
Spain	130 (27.4)	119 (25.4)
Italy	70 (14.7)	69 (14.7)
Hungary	70 (14.7)	68 (14.5)
Netherlands	67 (14.1)	66 (14.1)
Germany	55 (11.6)	58 (12.4)
Israel	47 (9.9)	48 (10.2)
Poland	30 (6.3)	34 (7.2)
Croatia	6 (1.3)	7 (1.5)
HLA risk group — no./total no. (%)‡		
1	80/462 (17.3)	49/449 (10.9)
2	46/462 (10.0)	42/449 (9.4)
3	199/462 (43.1)	218/449 (48.6)
4	29/462 (6.3)	37/449 (8.2)
5	108/462 (23.4)	103/449 (22.9)
First-degree relatives with celiac disease — no. (%)		
No. of relatives		
1	431 (90.7)	432 (92.1)
2	42 (8.8)	32 (6.8)
≥3	2 (0.4)	5 (1.1)
Type of relative		
Mother only	200 (42.1)	207 (44.1)
1 sibling	183 (38.5)	184 (39.2)
Father only	48 (10.1)	41 (8.7)
Mother and ≥1 sibling	23 (4.8)	23 (4.9)
>1 sibling but neither parent	12 (2.5)	7 (1.5)
Father and ≥1 sibling	9 (1.9)	5 (1.1)
Mother and father	0	2 (0.4)

* The characteristics of the children were similarly distributed between the intervention groups ($P<0.05$), with the exception of homozygosity for HLA-DQ2 ($P=0.05$).
† Data included a pair of healthy twins.
‡ Data on the HLA risk group were available for 911 of 944 children, with HLA typing performed by means of single-nucleotide polymorphisms (SNPs) on the basis of the tag-SNP approach.[8] The HLA risk groups were defined as follows: group 1 included DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); group 2 DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); group 3 DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); group 4 DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and group 5 DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); "other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7. For the remaining 33 children, the status with regard to HLA-DQ2 and HLA-DQ8 positivity was determined by means of the Eu-Gen Risk test (Eurospital), with no information provided regarding the HLA risk group.

of the intervention on the development of celiac disease (P=0.70 [for exclusive breast-feeding] and P=0.83 [for nonexclusive breast-feeding] for interaction; hazard ratios are provided in Table S3 in the Supplementary Appendix).

The cumulative incidence of celiac disease was significantly higher among girls randomly assigned to gluten than among those randomly assigned to placebo: at 3 years of age, the incidence was 8.9% in the gluten group versus 5.5% in the placebo group (hazard ratio 1.99; 95% CI, 1.09 to 3.65; P=0.02) (Fig. 2B). This difference was not seen among boys, with frequencies of 3.2% in the gluten group and 3.6% in the placebo group (hazard ratio, 0.62; 95% CI, 0.31 to

Figure 2 (facing page). Cumulative Incidence of Celiac Disease.

A total of 75 of 80 children received a diagnosis of celiac disease before 5 years of age. The cumulative incidence of celiac disease in the gluten group versus the placebo group at 3, 4, and 5 years of age was as follows: 5.9% versus 4.5%, 10.3% versus 7.3%, and 13.5% versus 10.6%, respectively (Panel A). The cumulative incidence among 454 girls in the gluten group and the placebo group was as follows: 8.9% versus 5.5%, 15.1% versus 8.5%, and 21.0% versus 8.5%, respectively (Panel B). The cumulative incidence among 490 boys was as follows: 3.2% versus 3.6%, 5.9% versus 3.6%, and 7.0% versus 13.4%, respectively (Panel C). The data in Panels B and C show a significant interaction between sex and intervention (P=0.01). The insets show the same data on an expanded y axis.

Table 2. Distribution of Symptoms and Celiac Disease–Associated Antibodies in 94 Children with Suspected Celiac Disease Who Underwent Diagnostic Small-Bowel Biopsies.*

Variable	Eventual Diagnosis				
	Celiac Disease (77 biopsies)	Potential Celiac Disease (5 biopsies)†	Unclear Diagnosis (2 biopsies)	No Celiac Disease (17 biopsies)	Total (101 biopsies)
Symptoms as indication for biopsy (no.)	52	0	2	13	67
Elevated antibody level as indication for biopsy (no.)‡					
TG2A	77	5	0	0	82
Antigliadin antibodies	12	0	0	6	18
Marsh classification of findings in small-bowel biopsies (no.)§					
0	0	4	0	13	17
1	0	1	0	1	2
2	3¶	0	2	3	8
3A	18	0	0	0	18
3B	24	0	0	0	24
3C	32	0	0	0	32
4	0	0	0	0	0

* One child with an elevated TGA2 level underwent biopsy three times: the histologic findings were normal the first two times but were compatible with celiac disease the last time. Five children underwent small-bowel biopsies twice. The first time, all had normal histologic findings; the second time, two children had normal histologic findings (none had potential celiac disease), and three received a diagnosis of celiac disease.

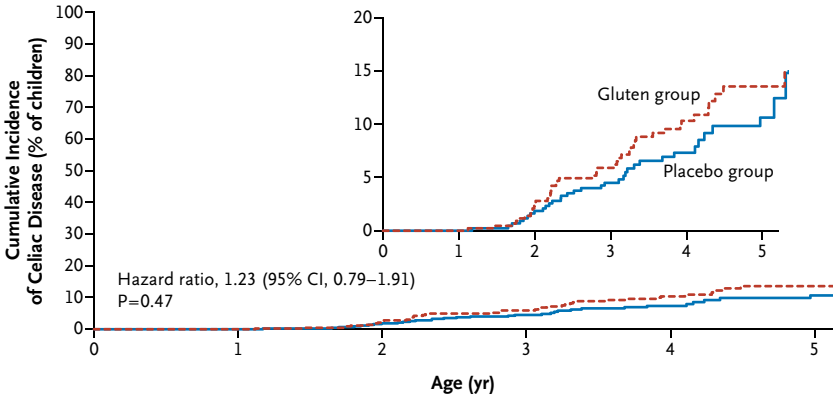
† Potential celiac disease was defined as an elevated level of anti–transglutaminase type 2 antibodies (TG2A) and normal histologic findings in the small bowel.

‡ An elevated serum level of IgA TG2A was defined as a level of 6 U per milliliter or more (or in the case of IgA deficiency, an IgG TG2A level of ≥10 U per milliliter). An elevated antigliadin antibody level was defined as a level of more than 50 U per milliliter (or in the case of IgA deficiency, an IgG TG2A level of ≥17 U per milliliter) on three occasions during a 3-month period or a level of more than 17 U per milliliter that was clearly increasing in two tests performed during a 3-month period.

§ Findings on small-bowel biopsies were assessed according to the Marsh classification,[30] on a scale of 0 to 4, with classes 0 and 1 being not characteristic of celiac disease, class 2 being compatible with celiac disease only with a concomitant elevated TG2A level, classes 3A to 3C being characteristic of celiac disease (with higher letter grades indicating more villous atrophy), and class 4 being characteristic of refractory celiac disease.

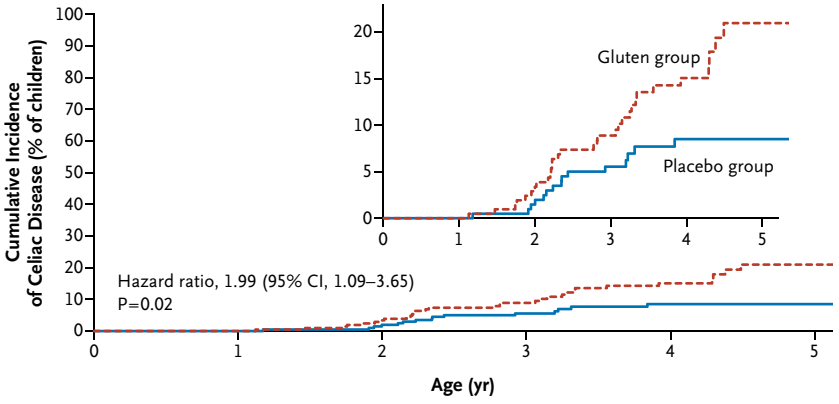
¶ Three children had a concomitant elevated TG2A level, as compared with the other five children with a Marsh classification of 2.[1]

A All Children



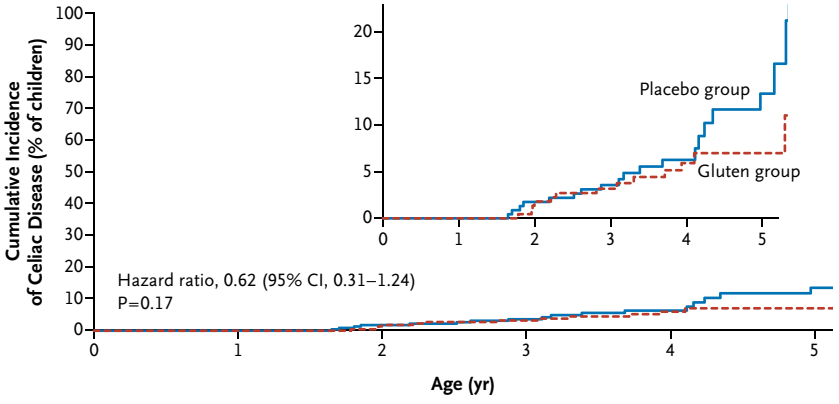
No. of Events/No. at Risk						
Gluten group	475	0/440	11/416	14/350	13/214	5/92
Placebo group	469	0/444	8/417	11/356	8/222	5/96

B Girls



No. of Events/No. at Risk						
Gluten group	228	0/213	7/199	11/165	9/99	4/32
Placebo group	226	0/209	4/196	7/170	4/109	0/51

C Boys



No. of Events/No. at Risk						
Gluten group	247	0/227	4/217	3/185	4/115	1/60
Placebo group	243	0/235	4/221	4/186	4/113	5/45

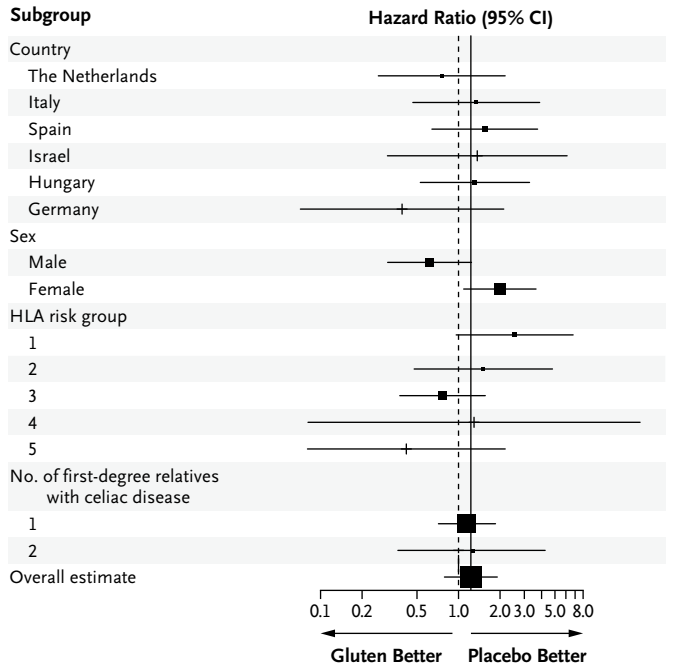


Figure 3. Effect of Intervention Assignment at 16 to 24 Weeks of Age on the Development of Celiac Disease in 944 Children from High-Risk Families.

Female sex was the only factor to significantly favor placebo (P=0.02). The HLA risk groups were defined as follows: group 1 included DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); group 2 DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); group 3 DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4-DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); group 4 DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and group 5 DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5-DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); "other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7. No statistics were computed for children from Poland (64 children) and Croatia (13), or for children with three or more first-degree relatives with celiac disease (7) because of the low number of children with celiac disease in these groups. The black boxes represent the hazard ratio with 95% confidence intervals (horizontal lines); the size of each box is proportional to the size of the corresponding subgroup. The overall estimate is represented by the solid vertical line; a dashed vertical line representing no effect is also shown.

1.24; P=0.17; P=0.01 for interaction of sex and intervention) (Fig. 2C). No factors other than sex were found to significantly influence the effect of the intervention on the development of celiac disease (Fig. 3, and Table S3 in the Supplementary Appendix).

The results of the primary per-protocol analysis were similar to those of the intention-to-treat analysis (see the Supplementary Appendix). The cumulative incidence of celiac disease seropositivity (positive TG2A, positive antigliadin antibodies, or both on two occasions during a 3-month period) did not differ significantly between the gluten group and the placebo group (7.0% [95% CI, 4.7 to 9.4] and 5.7% [95% CI, 3.5 to 7.9], respectively; hazard ratio, 1.14 [95% CI, 0.76 to 1.73]; P=0.53) (Table 3, and Fig. S3 in the Supplementary Appendix). Although elevated levels of TG2A were not found in any of the participants at 6 months of age, transient antigliadin antibody levels of more than 17 U per milliliter were observed in 59 children in the gluten group and 2 in the placebo group. This elevation was not predictive of celiac disease, which developed in only 8 of these children, all in the gluten group.

DISCUSSION

Our results indicate that the early introduction (at 16 weeks of age) of small quantities of gluten did not reduce the risk of celiac disease at 3 years of age in genetically predisposed children from high-risk families; therefore, our results do not support the protective effect that we had hypothesized. In addition, we found that breast-feeding, whether exclusive or not, did not have a significant effect on the frequency of celiac disease among these children. In prespecified secondary analyses, we observed an association between the early gluten intervention and celiac disease in girls but not in boys. We did not find significant effects in the other subgroups examined, and the significant finding in girls may be due to chance or to the larger number of girls with HLA-DQ2 homozygosity who were randomly assigned to gluten rather than to placebo (Table S4 in the Supplementary Appendix). Owing to the small number of children in the different HLA risk groups stratified according to sex, we cannot resolve this issue.

The higher frequency of celiac disease among girls than among boys after early exposure to gluten may be related to the well-known increased risk of celiac disease among women,[13,31] but it appears too early in life to be related to the protective effect of androgens for autoimmunity.[32] The gut microbiota may also play a role in this sexual dimorphism, as was shown recently for type 1 diabetes in rodents, in which hormones and microbes together trigger protective pathways.[32,33] Our results also show prospectively the effect of HLA-DQ2 homozygosity on the risk of celiac disease in early childhood.

In general, we found no association between

Table 3. Antibody Elevations and Diagnosis of Celiac Disease According to Intervention Assignment.*

Variable	Cumulative Incidence		P Value†	Hazard Ratio (95% CI)
	Gluten (N=475)	Placebo (N=469)		
	%			
Elevated antibody level at 6 mo of age				
Antigliadin	12.4	0.4	<0.001	
TG2A	0.0	0.0	NA	
Elevated level of TG2A or antigliadin antibody			0.53	1.14 (0.76–1.73)
At 1 yr of age	0.9	0.0		
At 2 yr of age	3.2	2.1		
At 3 yr of age	7.0	5.7		
At 4 yr of age	11.5	9.5		
At 5 yr of age	14.0	12.1		
Celiac disease			0.47	1.23 (0.79–1.91)
At 1 yr of age	0.0	0.0		
At 2 yr of age	2.6	1.9		
At 3 yr of age	5.9	4.5		
At 4 yr of age	10.3	7.3		
At 5 yr of age	13.5	10.6		

* NA denotes not applicable.
† The P value for the elevated antibody level at 6 months of age was calculated by means of Fisher's exact test, and the other P values were calculated by means of the log-rank test.

the early development of celiac disease and the presence of the disease in one or both parents, but this finding should be interpreted with caution, given the small number of fathers with celiac disease in our cohort (105 of 944 fathers). Possible explanations for the small number of affected fathers are the tendency for mothers to be more involved in research projects[34] and the higher frequency of celiac disease among women.[13]

Contrary to previous reports,[35,36] our data show that determining the TG2A level, but not the level of antigliadin antibodies, is useful in the assessment of the presence of celiac disease in very young children. In fact, we found that symptoms were not prognostic for celiac disease (Table 2), indicating that the early determination of the TG2A level in genetically predisposed children may offer an opportunity for early diagnosis.[37]

The strength of our study lies in its design as a randomized, double-blind, placebo-controlled trial evaluating a food intervention in a high-risk birth cohort, with comprehensive follow-up. The cases of celiac disease were assessed in an identical way, minimizing the risk of bias. Nonetheless, our study has some limitations. It may be argued that we introduced gluten in a rather artificial way, since 100 mg is approximately 2% of the amount normally introduced at weaning.[29] Nevertheless, this quantity has been shown previously to cause histologic damage in the intestines of patients with celiac disease.[38] After our gluten intervention, levels of antigliadin antibodies were transiently elevated in 59 children at 6 months of age, showing that 100 mg of gluten can indeed be immunogenic. Our power calculation was based on the assumption of a cumulative incidence of celiac disease of 10% by 3 years of age. We found that the actual mean frequency at this age was half the assumed frequency and that it strongly depended on sex and HLA haplotype. The confidence intervals for the hazard ratio for the effect of the intervention on celiac disease ranged from 0.79 to 1.91, indicating that we were not able to rule out a protective effect smaller than 21% or a harmful effect as large as 91%.

Our findings contrast with those from obser-

vational studies suggesting that the introduction of gluten between the ages of 4 months and 6 months represents a window of opportunity for preventing celiac disease.[23,24] Much of the information on infant feeding and the risk of celiac disease has been obtained from studies of the Swedish celiac disease epidemic, which started in the mid-1980s[21] and was related to the introduction of an increased amount of gluten after the age of 6 months, when breast-feeding became less common.[9,22,23,39] However, data regarding the timing of gluten introduction in relation to breast-feeding, as well as the amount of gluten, were obtained retrospectively. Our results also contrast with recent findings in a prospective cohort of young children from the general population in Norway.[25] However, that study investigated only clinically diagnosed celiac disease, with probable underreporting of celiac disease, since most cases are not clinically recognized. Whereas the observations in the Swedish and Norwegian cohorts are based on the general population from single countries, our results are derived from a study population comprising children from high-risk families in seven European countries and Israel. Observational studies involving children with an increased risk of type 1 diabetes (positive for HLA-DQ2 or HLA-DQ8) have had controversial results. Although the results of a study conducted in the United States support the early introduction of gluten at 4 to 6 months of age,[24] the age at gluten introduction did not influence the risk of celiac disease autoimmunity in a prospective German birth cohort.[40]

In conclusion, this randomized trial did not show the hypothesized benefit of early exposure to small quantities of gluten with regard to reducing the incidence of celiac disease among children from high-risk families. In addition, we did not observe a reduced risk of celiac disease associated with the maintenance of breast-feeding at the time of gluten introduction. The present European guidelines recommend the introduction of small amounts of gluten gradually while the child is breast-fed and the avoidance of both the early (<4 months) or late (>7 months) introduction of gluten.[41] Our results do not provide evidence to support these guidelines or any specific feeding recommendation with respect to the timing of gluten introduction for infants at risk for celiac disease.

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APPENDIX

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REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012;54:136-60. [Erratum, J Pediatr Gastroenterol Nutr 2012;54:572.]
2. Auricchio S, Mazzacca G, Tosi R, Visakorpi J, Maki M, Polanco I. Coeliac disease as a family condition: identification of asymptomatic patients within family groups. Gastroenterology 1988;1:25-31.
3. Babron MC, Nilsson S, Adamovic S, et al. Meta and pooled analysis of European coeliac disease data. Eur J Hum Genet 2003;11:828-34.
4. Bourgey M, Calcagno G, Tinto N, et al. HLA related genetic risk for coeliac disease. Gut 2007;56:1054-9.
5. Collin P, Kaukinen K. Serologic screening for coeliac disease in risk groups: is once in the lifetime enough? Dig Liver Dis 2008;40:101-3.
6. Goldberg D, Kryszak D, Fasano A, Green PH. Screening for celiac disease in family members: is follow-up testing necessary? Dig Dis Sci 2007;52:1082-6.
7. Lionetti E, Castellaneta S, Pulvirenti A, et al. Prevalence and natural history of potential celiac disease in at-family-risk infants prospectively investigated from birth. J Pediatr 2012;161:908-14.
8. Monsuur AJ, de Bakker PI, Zhernakova A, et al. Effective detection of human leukocyte antigen risk alleles in celiac disease using tag single nucleotide polymorphisms. PLoS One 2008;3(5): e2270.
9. Myléus A, Ivarsson A, Webb C, et al. Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. J Pediatr Gastroenterol Nutr 2009;49: 170-6.
10. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. J Exp Med 1989;169:345-50.
11. Green PH, Jabri B. Coeliac disease. Lancet 2003;362:383-91.
12. Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Hum Immunol 2003;64:469-77.
13. Mearin ML. Celiac disease among children and adolescents. Curr Probl Pediatr Adolesc Health Care 2007;37:86-105.
14. Sollid LM, Qiao SW, Anderson RP, Gianfrani C, Koning F. Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. Immunogenetics 2012;64:455-60.
15. Biagi F, Corazza GR. Mortality in celiac disease. Nat Rev Gastroenterol Hepatol 2010;7:158-62.
16. van Doorn RK, Winkler LM, Zwinderman KH, Mearin ML, Koopman HM. CDDUX: a disease-specific health-related quality-of-life questionnaire for children with celiac disease. J Pediatr Gastroenterol Nutr 2008;47:147-52.
17. Hogen Esch CE, Csizmadia GD, van Hoogstraten IM, Schreurs MW, Mearin ML, von Blomberg BM. Childhood coeliac disease: towards an improved serological mass screening strategy. Aliment Pharmacol Ther 2010;31:760-6.
18. Shamir R, Hernell O, Leshno M. Cost-effectiveness analysis of screening for celiac disease in the adult population. Med Decis Making 2006;26:282-93.
19. Hogen Esch CE, Rosén A, Auricchio R, et al. The PreventCD Study design: towards new strategies for the prevention of coeliac disease. Eur J Gastroenterol Hepatol 2010; 22:1424-30.
20. Troncone R, Auricchio R, Granata V. Issues related to gluten-free diet in coeliac disease. Curr Opin Clin Nutr Metab Care 2008;11:329-33.
21. Ivarsson A, Persson LA, Nyström L, et al. Epidemic of coeliac disease in Swedish children. Acta Paediatr 2000;89:165-71.
22. Ivarsson A, Hernell O, Stenlund H, Persson LA. Breast-feeding protects against celiac disease. Am J Clin Nutr 2002;75:914-21.
23. Ivarsson A, Myléus A, Norström F, et al. Prevalence of childhood celiac disease and changes in infant feeding. Pediatrics 2013;131(3):e687-e694.
24. Norris JM, Barriga K, Hoffenberg EJ, et al. Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. JAMA 2005;293:2343-51.
25. Stødal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132(5):e1202-e1209.
26. Akobeng AK, Ramanan AV, Buchan I, Heller RF. Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies. Arch Dis Child 2006;91:39-43.
27. Szajewska H, Chmielewska A, Pieścik-Lech M, et al. Systematic review: early infant feeding and the prevention of coeliac disease. Aliment Pharmacol Ther 2012;36: 607-18.
28. Revised criteria for diagnosis of coeliac disease: report of Working Group of European Society for Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990;65:909-11.
29. Hopman EG, Kiefte-de Jong JC, le Cessie S, et al. Food questionnaire for assessment of infant gluten consumption. Clin Nutr 2007;26:264-71.
30. Villanacci V, Ceppa P, Tavani E, Vindigni C, Volta U. Coeliac disease: the histology report. Dig Liver Dis 2011;43:Suppl 4: S385-S395.
31. Markle JG, Fish EN. SeXX matters in immunity. Trends Immunol 2014;35:97-104.
32. Yurkovetskiy L, Burrows M, Khan AA, et al. Gender bias in autoimmunity is influenced by microbiota. Immunity 2013; 39:400-12.
33. Markle JG, Frank DN, Mortin-Toth S, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 2013;339: 1084-8.
34. Phares V, Lopez E, Fields S, Kamboukos D, Duhig AM. Are fathers involved in pediatric psychology research and treatment? J Pediatr Psychol 2005;30:631-43.
35. Ascher H, Hahn-Zoric M, Hanson LA, Kilander AF, Nilsson LA, Tlaskalová H. Value of serologic markers for clinical diagnosis and population studies of coeliac disease. Scand J Gastroenterol 1996;31: 61-7.
36. Lagerqvist C, Dahlbom I, Hansson T, et al. Antigliadin immunoglobulin A best in finding celiac disease in children younger than 18 months of age. J Pediatr Gastroenterol Nutr 2008;47:428-35.
37. van Koppen EJ, Schweizer JJ, Csizmadia CG, et al. Long-term health and quality-of-life consequences of mass screening for childhood celiac disease: a 10-year follow-up study. Pediatrics 2009;123(4): e582-e588.
38. Catassi C, Rossini M, Rätsch IM, et al. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. Gut 1993;34: 1515-9.
39. Ivarsson A, Högberg L, Stenhammar L. The Swedish Childhood Coeliac Disease Working Group after 20 years: history and future. Acta Paediatr 2010;99: 1429-31.
40. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA 2003; 290:1721-8.
41. Agostoni C, Decsi T, Fewtrell M, et al. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2008; 46:99-110.