ORIGINAL ARTICLE

Introduction of Gluten, HLA Status, and the Risk of Celiac Disease in Children

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ABSTRACT

BACKGROUND

The relationship between the risk of celiac disease and both the age at which gluten is introduced to a child's diet and a child's early dietary pattern is unclear.

METHODS

We randomly assigned 832 newborns who had a first-degree relative with celiac disease to the introduction of dietary gluten at 6 months (group A) or 12 months (group B). The HLA genotype was determined at 15 months of age, and serologic screening for celiac disease was evaluated at 15, 24, and 36 months and at 5, 8, and 10 years. Patients with positive serologic findings underwent intestinal biopsies. The primary outcome was the prevalence of celiac disease autoimmunity and of overt celiac disease among the children at 5 years of age.

RESULTS

Of the 707 participants who remained in the trial at 36 months, 553 had a standard-risk or high-risk HLA genotype and completed the study. At 2 years of age, significantly higher proportions of children in group A than in group B had celiac disease autoimmunity (16% vs. 7%, P=0.002) and overt celiac disease (12% vs. 5%, P=0.01). At 5 years of age, the between-group differences were no longer significant for autoimmunity (21% in group A and 20% in group B, P=0.59) or overt disease (16% and 16%, P=0.78 by the log-rank test). At 10 years, the risk of celiac disease autoimmunity was far higher among children with high-risk HLA than among those with standard-risk HLA (38% vs. 19%, P=0.001), as was the risk of overt celiac disease (26% vs. 16%, P=0.05). Other variables, including breast-feeding, were not associated with the development of celiac disease.

CONCLUSIONS

Neither the delayed introduction of gluten nor breast-feeding modified the risk of celiac disease among at-risk infants, although the later introduction of gluten was associated with a delayed onset of disease. A high-risk HLA genotype was an important predictor of disease. (Funded by the Fondazione Celiachia of the Italian Society for Celiac Disease; CELIPREV ClinicalTrials.gov number, NCT00639444.)

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Celiac disease is a systemic immune-mediated disorder caused by the ingestion of gluten-containing grains (wheat, rye, and barley) in genetically susceptible persons. It is one of the most common lifelong disorders, affecting approximately 1% of the population in Europe and North America[1,2]; the prevalence of this disease is higher among persons who have first-degree relatives with celiac disease (10 to 15%).[3,4] The prevalence of celiac disease has increased in developed countries over recent decades; this finding points to the role of one or more possible environmental triggers other than gluten.[5]

Genetic background plays a key role in the predisposition to celiac disease. The HLA-DQ2 (DQA1*0501-DQB1*0201) haplotype is expressed in the majority of affected patients (90%), the DQ8 haplotype (DQA1*0301-DQB1*0302) is expressed in 5%, and 5% carry at least one of the two DQ2 alleles (usually the DQB1*0201). An increased risk of celiac disease has been observed among persons who carry two DQB1*02 alleles.[6,7] Gluten is required to trigger the disease, but the interplay between genetic and environmental factors regulating the balance between tolerance and immune response to gluten is still poorly understood. It has been hypothesized that intestinal infections, the amount and quality of ingested gluten, the composition of intestinal microbiota, and infant-feeding practices are all possible triggers of the switch from tolerance to an immune response to gluten.[1]

The introduction of gluten at 6 months of age is a long-standing practice.[8] Despite the fact that the "gluten at 6 months" rule is deeply rooted in many developed countries, the optimal timing of the introduction of gluten in the infant's diet has not been rigorously tested. Many clinicians advise that the introduction of gluten to the diet of infants who have a familial risk of the disease should be delayed. Depending on timing, this delay may permit the maturation of the small intestinal barrier and the mucosal immune response.[9,10] However, investigations of the epidemic of celiac disease that occurred in Sweden during the 1980s and 1990s indicate that the introduction of a small amount of gluten during breast-feeding of infants between 4 and 6 months of age reduces the risk of disease.[11,12] Studies involving infants at genetic risk for type 1 diabetes suggest that the risk of type 1 diabetes or celiac disease is increased among infants who began to receive gluten either before 4 months or after 7 months of age; this provides support for the view that there is a window of time, between 4 and 7 months, during which the introduction of gluten might facilitate induction of tolerance.[13-15]

To clarify the relationship between the age at which gluten is introduced to a child's diet and the risk of celiac disease, we undertook the Risk of Celiac Disease and Age at Gluten Introduction (CELIPREV) trial, a multicenter, prospective intervention trial comparing early and delayed introduction of gluten to the diet of infants with a familial risk of celiac disease, and we followed these children from birth to at least 5 years of age.

METHODS

PATIENTS AND STUDY DESIGN

Newborns who had a familial risk of celiac disease (i.e., newborns who had at least one first-degree relative with celiac disease) were recruited at 20 centers in Italy between 2003 and 2008. Infants were assigned on the basis of block randomization to one of two groups: those in group A were assigned to the introduction of food containing gluten (pasta, semolina, and biscuits) at 6 months of age, and those in group B were assigned to the introduction of food containing gluten at 12 months of age. The primary outcome was the prevalence of celiac disease autoimmunity and of overt celiac disease among patients with a standard-risk or high-risk HLA genotype according to trial group at 5 years of age.

At 12 months of age, all children began to receive a normal diet containing gluten. Interviews were conducted to obtain information on the diet and intestinal infections during the first year of life. The daily intake of cereal containing gluten (wheat, rye, and barley) was assessed by means of a 24-hour dietary-recall questionnaire, and daily gluten intake was calculated as the sum of grams of protein obtained from gluten-related grains multiplied by 0.8.[16] A workup for celiac disease was performed at 15 months of age (IgA antitransglutaminase type 2 [TGA2], IgA antigliadin antibodies, total IgA, and HLA-DQ2 and HLA-DQ8 genotype), at 24 months (TGA2 and antigliadin antibodies), and at 3, 5, 8, and 10 years (TGA2). In children with IgA deficiency (IgA <5 mg per deciliter), we tested for the presence of IgG antigliadin antibodies. Children with positive serologic results were recalled for repeti-

tion of the test and determination of the presence of endomysial antibodies. A small-bowel biopsy was recommended for children with seropositivity for celiac disease autoimmunity (i.e., positive results for IgA TGA2 and endomysial antibodies, positive results for IgG antigliadin antibodies with IgA deficiency, or positive results for IgA antigliadin antibodies in children younger than 2 years of age). We defined overt celiac disease as celiac disease autoimmunity and a Marsh classification of 2 or 3 at small-bowel biopsy (on a scale of 0 to 3, with higher scores indicating villous atrophy), and potential celiac disease as celiac disease autoimmunity and a Marsh classification of 0 or 1. Children with overt celiac disease began to receive a gluten-free diet, and those with potential celiac disease continued to receive a normal diet unless they had symptoms. Findings from the 2-year follow-up of a subgroup of children with potential celiac disease were reported in a previous study.[17]

The study protocol was approved by the institutional review board at each participating center. Written informed consent was obtained from the parents or guardians of the children.

All the authors vouch for the accuracy of the data and analyses reported and the fidelity of the study to the protocol. The study protocol is available with the full text of this article at NEJM.org.

HLA GENOTYPING

The detection of HLA alleles was performed with the use of the DQ-CD Typing Plus kit (BioDiagene), and on the basis of this assessment, the children were classified as having no risk of celiac disease (the absence of HLA-DQ2 and HLA-DQ8), a standard risk of celiac disease (a single or double copy of the DQB1*02 allele associated with DQA1 alleles different from the DQA1*05, or a single DQ2 [DQA1*05-DQB1*02] either in *cis* or *trans* position, or DQ8 [DQA1*03-DQB1*0302/0305] haplotypes), or a high risk of celiac disease (homozygosity for DQA1*05-DQB1*02 or DQA1*05-DQB1*02-DQA1*0201-DQB1*02).[18,19]

SEROLOGIC ASSAYS

All serum samples were kept frozen at –20°C until analysis in a single laboratory at Udine Hospital, Udine, Italy. Serum IgA TGA2 was measured by means of an enzyme-linked immunosorbent assay (ELISA) with the use of a commercial kit (Menarini Diagnostics). More than 20 arbitrary units indicated a positive result. IgA antigliadin antibodies and IgG antigliadin antibodies were measured by means of ELISA with the use of a commercial kit (Menarini Diagnostics); more than 15 arbitrary units indicated a positive result. Endomysial antibodies were detected by means of indirect immunofluorescence, with the use of monkey esophagus as substrate (a titer of 1:10 or higher that resulted in a positive reaction was considered to be positive), and total serum IgA was measured by means of nephelometry.

Table 1. Characteristics of the Participants.*

Variable	Group A (N=297)	Group B (N=256)
Median age — yr	7.8	7.9
Female sex — no. (%)	146 (49.2)	128 (50.0)
First-degree relatives with celiac disease — no. (%)		
Type of relative		
Father	23 (7.7)	16 (6.2)
Mother	95 (32.0)	112 (43.8)
Brother	59 (19.9)	63 (24.6)
Sister	91 (30.6)	44 (17.2)
No. of relatives		
1	268 (90.2)	235 (91.8)
2	25 (8.4)	19 (7.4)
3	4 (1.3)	2 (0.8)
Breast-fed — no. (%)	193 (65.0)	178 (69.5)
Duration of breast-feeding — mo	5.9±5.4	6.4±6.4
Breast-fed during introduction of gluten — no. (%)†	84 (28.3)	29 (11.3)
Intake of gluten — g/day		
At 9 mo†	3.2±1.5	0
At 15 mo	6.5±2	6.8±2
Age at introduction of gluten — mo†	6.1±0.9	12.2±0.9
HLA genotype — no. (%)		
Standard risk	265 (89.2)	230 (89.8)
High risk	32 (10.8)	26 (10.2)

* Plus–minus values are means ±SD. The difference between the groups was assessed with the use of Student's t-test and the chi-square test.
† P<0.001 for the comparison between the groups.

DIAGNOSIS OF OVERT CELIAC DISEASE

Small-bowel biopsies were performed by means of upper endoscopy, and at least four specimens were obtained from the bulb and the descending part of the duodenum. The ratio of villous height to crypt depth was measured, and a ratio of 2 or

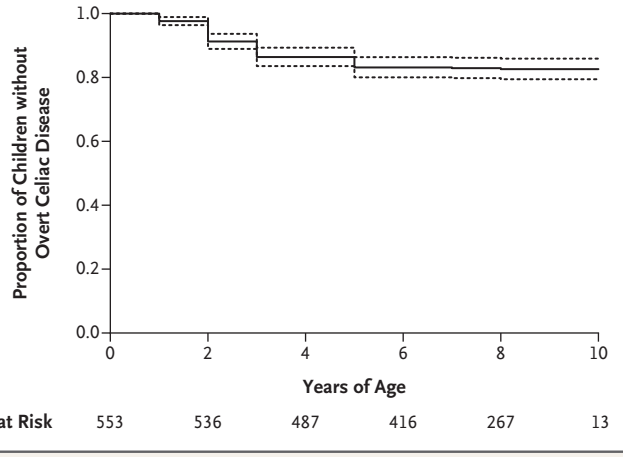

Figure 1. Kaplan–Meier Estimates of Overt Celiac Disease in Children with High-Risk HLA Genotypes, According to Age.
Dashed lines represent 95% confidence intervals.

more was considered normal. Intraepithelial lymphocytosis was defined as more than 25 intraepithelial lymphocytes per 100 epithelial cells. Lesions in the small intestine were graded at the coordinating center in Ancona, Italy, according to the Marsh classification.[20] The graders were not informed of the group assignments.

STATISTICAL ANALYSIS

The analysis included all randomly assigned children classified according to their assigned diet. Participants with missing data on serologic outcomes were included in the final analysis if at least one serologic result was available at 36 months of follow-up or thereafter; participants who were lost to follow-up before 36 months or for whom we had no serologic data were excluded from the analysis.

Data are expressed as means ±SD. Proportions were compared with the use of the chi-square test with Yates' correction for continuity or Fisher's exact test as appropriate; comparison of continuous variables was performed with the use of Student's t-test. Kaplan–Meier curves were plotted for the primary end point (i.e., the risk of celiac disease according to age and trial group) and the secondary end point (i.e., the risk of celiac disease according to trial group in a single prespecified subgroup of interest [children with high-risk HLA genotypes]). Differences between these curves were assessed with the use of log-rank tests and proportional-hazards models. All differences were considered to be statistically significant at a 5% probability level, and all reported P values are two-sided. Risk models were developed with the use of decision-tree induction from class-labeled training records (i.e., the training set was composed of records in which one attribute was the class label [or dependent variable] and the remaining attributes were the predictor variables; the individual records are the tuples for which the class label is known), as previously described.[17,21] Statistical analysis was performed with the use of tools for survival analysis and recursive partitioning analysis within the R system.

RESULTS

PATIENTS

Data on enrollment and study-group assignments are shown in Figure S1 in the Supplementary Appendix, available at NEJM.org. After exclusion of 125 patients who dropped out, the cohort included 707 infants (379 in group A and 328 in group B). There were 351 girls (49.6%), and the median age of the cohort was 7.9 years (range, 5.2 to 10.6) in October 2013, when the study was terminated. The primary reason for the imbalance between groups with respect to the number of participants in this multicenter study was our inability to fully control the higher patient dropout rate in group B soon after randomization. By the end of the study, all children were at least 5 years of age, 75% were 7 years of age, 49% were 8 years of age, and 16% were 10 years of age. Of the 707 infants tested for HLA status, 154 were in the HLA no-risk group (21.8%) and were excluded from further analysis. The final study group included 553 children who were positive for HLA-DQ2, HLA-DQ8, or both (Table 1).

We obtained serologic data from all 553 children at 15 months of age and from 536 children at 24 months of age (97%), 487 children at 36 months of age (88%) (by which point 66 of the 553 children in the final study group had overt celiac disease and were therefore not tested for celiac disease autoimmunity), 451 children at 5 years of age (82%), 373 children at 7 years of age (67%), 267 children at 8 years of age (48%), and 89 children at 10 years of age (16%). Overall, 122 children had positive serologic results, but only 117 fulfilled the criteria for small-bowel biopsy; the other 5 had isolated, low-titer TGA2 positivity. Of the 117 children with celiac disease autoimmunity, 113 were positive for TGA2 and endomysial antibodies, 3 were positive for IgG antigliadin anti-

bodies with IgA deficiency, and 1 was positive for IgA antigliadin antibodies. Of these 117 children, 112 underwent small-bowel biopsy; the parents of the remaining 5 children (all of whom were positive for TGA2 and endomysial antibodies) instituted a gluten-free diet. These 5 patients were all symptomatic, and their symptoms improved with the use of a gluten-free diet; therefore, they were included in the group of children with overt celiac disease. Of the 112 children who underwent biopsy of the small intestine, 86 had a Marsh classification of 2 or higher (5 children had a score of 2, 6 had a score of 3a, 33 had a score of 3b, and 42 had a score of 3c) and received a diagnosis of overt celiac disease. A total of 26 children had a Marsh classification of 0 (15 children) or 1 (11 children) and received a diagnosis of potential celiac disease. A 4-year follow-up assessment after the first biopsy showed that 19 of these 26 children had normalization of serum antibody levels, 2 had fluctuating antibody levels, and 3 had begun to receive a gluten-free diet on the basis of parental choice. The other 2 had undergone biopsy a second time and been found to have lesions with a Marsh classification of 3; they were therefore reclassified as having overt celiac disease.

STUDY OUTCOMES

Overall, at 10 years of age, among children with a familial risk of celiac disease, the prevalence of celiac disease autoimmunity was 16.5% (in 117 of 707 children) and the prevalence of overt celiac disease was 13.2%. At the age of 10 years, among children who were found to have a familial risk and a celiac disease–predisposing HLA genotype in infancy, the prevalence of celiac disease autoimmunity was 21.1% and the prevalence of overt celiac disease was 16.8% (Fig. 1). Celiac disease autoimmunity developed in 37.9% of children with high-risk HLA genotypes as compared with 19.2% of children with standard-risk HLA genotypes (hazard ratio, 0.5; 95% confidence interval [CI], 0.3 to 0.7; P=0.001) (Fig. S2A in the Supplementary Appendix). Overt celiac disease developed in 25.8% of children with high-risk HLA genotypes as compared with 15.8% of children with standard-risk HLA genotypes (hazard ratio, 0.6; 95% CI, 0.3 to 1.0; P=0.05) (Fig. S2B in the Supplementary Appendix).

Figure 2 shows the proportion of children in whom overt celiac disease and celiac disease autoimmunity developed, according to study group.

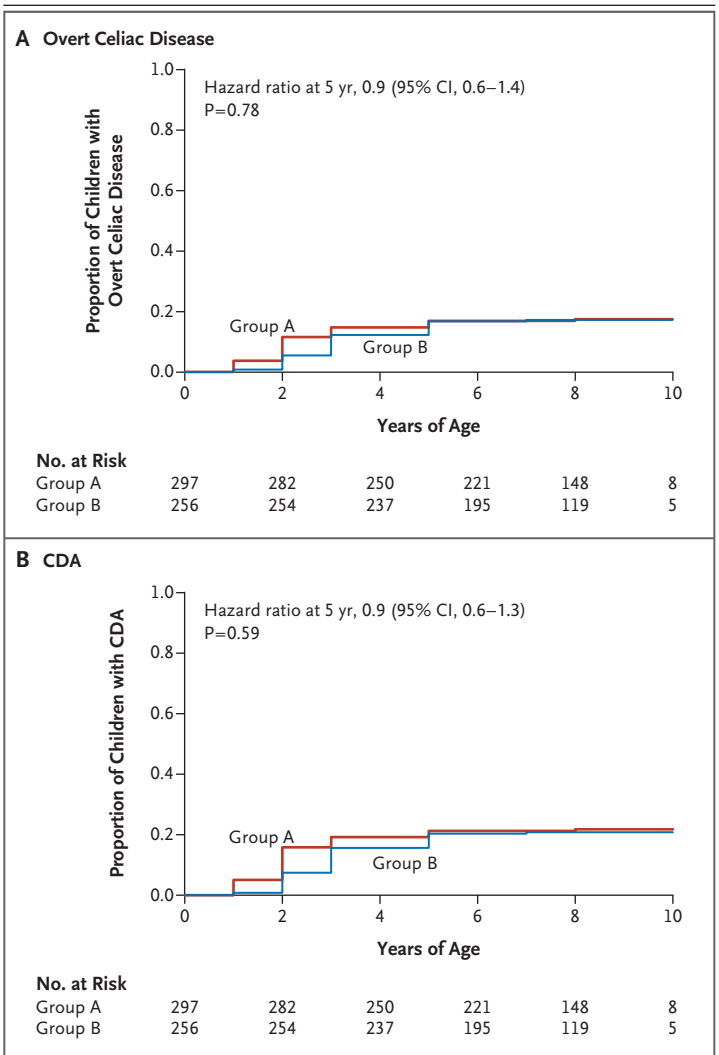

Figure 2. Kaplan–Meier Estimates of Celiac Disease Autoimmunity (CDA) and Overt Celiac Disease, According to Study Group.

At 2 years of age, the proportion of children with overt celiac disease was significantly higher in group A than in group B (12% vs. 5%, P=0.01). However, this difference had resolved by 5 years of age (16% vs. 16%; hazard ratio for the development of overt celiac disease, 0.9; 95% CI, 0.6 to 1.4; P=0.78) and was not observed at 8 or at 10 years of age (hazard ratio at 10 years, 0.9; 95% CI, 0.6 to 1.4; P=0.79). The median age at diagnosis of overt celiac disease was 26 months in group A and 34 months in group B (P=0.01).

There was a significantly greater prevalence of celiac disease autoimmunity in group A than in group B at 2 years of age (16% vs. 7%, P=0.002), and the difference had resolved by 5 years of age (21% and 20%, respectively; P=0.59). In children

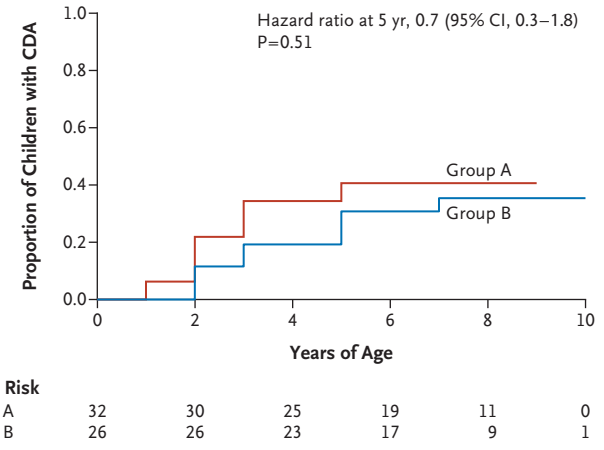

Figure 3. Kaplan–Meier Estimates of CDA in Children with a Celiac Disease–Predisposing HLA Genotype, According to Study Group.

with high-risk HLA alleles, the prevalence of celiac disease autoimmunity was greater in group A than in group B at all ages, but the difference was not significant (P=0.51) (Fig. 3).

The clinical characteristics of children with and those without celiac disease are shown in Table 2. During follow-up, celiac disease–related complications (i.e., autoimmune thyroid disease, type 1 diabetes, or both) did not develop in any of the children. The result of the decision-tree analysis for prediction of celiac disease autoimmunity and overt celiac disease is shown in Figures S3A and S3B in the Supplementary Appendix. The lowest-risk branch (leading to no celiac disease instead of celiac disease autoimmunity or overt celiac disease) was assigned to children with a standard-risk HLA genotype. None of the other variables studied had a significant effect in predicting the development of celiac disease. A Cox proportional-hazards model confirmed the decision-tree analysis of the contribution of each factor to the development of celiac disease (Tables S1A and S1B in the Supplementary Appendix).

DISCUSSION

We found that postponing the introduction of gluten until 12 months of age had no effect on the risk of the development of celiac disease in the long term. This result was consistent with the finding that delaying gluten exposure until the age of 12 months is safe but does not substantially reduce the risk of islet autoimmunity in children who are genetically at risk for type 1 diabetes.[22] Postponing the introduction of gluten had two potentially positive consequences. First, it delayed the development of celiac disease, which might reduce the negative effect of the disease on vulnerable organs such as the brain. Second, it reduced the prevalence, albeit nonsignificantly, of celiac disease autoimmunity at any age among children carrying the high-risk HLA genotype (Fig. 3). This finding provides support for Koning's HLA–gene dose model, which posits that targeted prevention of celiac disease in high-risk HLA infants prevents the development of uncontrolled T-cell responses to the high numbers of immunogenic HLA DQ2–gluten complexes.[23]

The concept of a window of gluten tolerance gained popularity after U.S. investigators reported in 2005 that at-risk children exposed to gluten at 4 to 6 months of age had a reduced risk of celiac disease, as compared with those exposed to gluten before 4 months or after 7 months of age, but the number of patients with biopsy-proven celiac disease in their study was small.[14] German infants with a familial risk of type 1 diabetes whose first dietary exposure to gluten occurred after the age of 6 months did not have an increased risk of celiac disease autoimmunity or islet autoantibodies.[24] A Norwegian study[25] that matched dietary data collected in a nationwide, prospective, questionnaire-based survey with the presence or absence of celiac disease showed only a minimally increased risk of celiac disease among infants introduced to gluten after 6 months of age, and adjusted analyses did not show an association between the early introduction of gluten (before 4 months of age) and an increased risk of celiac disease. The Norwegian study included only children with clinically diagnosed celiac disease, so its results do not necessarily apply to the overall population of persons with celiac disease. Our data showed no difference in the risk of celiac disease between children who were introduced to gluten at 6 months (during the open "window") and those who were introduced to gluten at 12 months (when the window was closed). Thus, our findings do not support the "window of tolerance" hypothesis.

Our study sheds light on other aspects of the relationship between infant nutrition and the risk of celiac disease. A protective role of breast-feeding has long been claimed, mostly based on a few

Table 2. Characteristics of Children with Celiac Disease Autoimmunity, Those with Overt Celiac Disease, and Those without Celiac Disease.*

Variable	Celiac Disease Autoimmunity (N=117)	Overt Celiac Disease (N=93)	No Celiac Disease (N=436)
Female sex — no. (%)	65 (55.6)	54 (58.1)	209 (47.9)
First-degree relatives with celiac disease — no. (%)			
Type of relative			
Father	9 (7.7)	8 (8.6)	30 (6.9)
Mother	46 (39.3)	34 (36.6)	161 (36.9)
Brother	17 (14.5)	14 (15.1)	85 (19.5)
Sister	33 (28.2)	24 (25.8)	121 (27.8)
No. of relatives			
1	105 (89.7)	80 (86.0)	397 (91.1)
2	10 (8.5)	11 (11.8)	33 (7.6)
3	2 (1.7)	2 (2.2)	6.0 (1.4)
Duration of breast-feeding — mo	5.6±6.3	6.0±6.5	5.8±5.8
Breast-fed during introduction of gluten — no. (%)	23 (19.7)	19.0 (20.4)	87.0 (20.0)
Intake of gluten at 15 mo — g/day	6.5±2.0	6.5±2	6.7±2.0
Age at introduction of gluten — mo	8.8±3.2	9.0±3.2	8.9±3.2
Introduction of gluten — no. (%)			
At 6 mo, group A	64 (54.7)	50 (53.8)	234 (53.7)
At 12 mo, group B	53 (45.3)	43 (46.2)	203 (46.6)
Enteritis, one or more episodes from 0 to 15 mo of age — no./total no. (%)†	5/58 (8.6)	3/42 (7.1)	29/249 (11.6)
Elevated antibody level at diagnosis — ×ULN			
TGA2 IgA	9.9±9.3	11.1±9.8	—
Antigliadin IgA	3.8±6.9	4.5±7.6	—
Symptoms of celiac disease — no. (%)			
Typical	46 (39.3)	46 (49.5)	—
Atypical	10 (8.5)	10 (10.8)	—
None	61 (52.1)	37 (39.8)	—
HLA genotype — no. (%)			
Standard risk‡	91 (77.8)	78 (83.9)	400 (91.7)
High risk	26 (22.2)	15 (16.1)	36 (8.3)

* Plus–minus values are means ±SD. The difference between the groups was tested by means of Student's t-test and the chi-square test. ULN denotes upper limit of the normal range.
† Data on enteritis were available for only 307 children. The diagnosis of enteritis was based on the presence of diarrhea associated with infectious disease.
‡ P=0.03 for the comparison between the group with overt celiac disease and the group with no celiac disease, and P<0.001 for the comparison between the group with celiac disease autoimmunity and the group with no celiac disease.

observational, retrospective studies,[26-29] which were summarized in a meta-analysis[30] and a systematic review.[31] These surveys did not clarify whether breast-feeding provides permanent protection against celiac disease or simply delays the onset of symptoms. The Norwegian study[25] not only did not support a protective effect of breast-feeding but also showed an unexpected association between breast-feeding that was prolonged beyond 12 months of age and an increased risk of celiac

disease. We did not detect an effect of breast-feeding on the development of celiac disease: the mean duration of breast-feeding was very similar for at-risk children among whom celiac disease developed and at risk-children among whom the disorder did not develop (5.6 and 5.8 months, respectively). We did not observe a protective effect of introducing gluten during breast-feeding, but our study was not designed to address this issue, which was evaluated in the European PreventCD study, which is reported by Vriezinga et al. in this issue of the *Journal*.[32]

We diagnosed celiac disease autoimmunity and overt celiac disease according to current guidelines.[33,34] The high prevalence of celiac disease autoimmunity by 5 years of age that we observed could indicate that the "epidemic" of celiac disease is continuing its upward trend, since it is known that celiac disease can develop in persons of any age, including the elderly.[5] Our estimate of the prevalence of celiac disease autoimmunity included patients with potential celiac disease, many of whom had a loss of antibodies with a normal diet, over the course of time. The long-term prognosis for children with transient celiac disease autoimmunity remains to be established; overt celiac disease could develop in some of them over time.[35]

It is possible that we underestimated the number of children in whom celiac disease developed because of incomplete follow-up, but overt celiac disease developed in the large majority of children within the first 5 years of life, and in 80% of those in whom celiac disease developed, it did so during the first 3 years. We therefore suggest that efficient screening for celiac disease may be carried out by testing school-age children. Our results support early determination of the HLA-DQ haplotype in infants with a familial risk of celiac disease, not only to exclude infants who are negative for HLA-DQ2 and HLA-DQ8 from further investigation but also to identify those with two copies of the HLA-DQ2 allele, who require close monitoring because of the high risk of disease[36] (38% in our at-risk cohort).

The high-risk HLA genotype was the only factor that was significantly associated with the development of celiac disease autoimmunity and overt celiac disease in the decision-tree analysis: other variables (type of relative with celiac disease, number of affected relatives, dietary pattern, and early intestinal infections) showed no association with disease risk.

In conclusion, our study suggests that celiac disease autoimmunity tends to develop in genetically predisposed children early in life, usually before the age of 5 years. Early dietary factors, particularly the child's age at the introduction of gluten, seem to play a minor role in the risk of the development of celiac disease. However, delaying the introduction of gluten in at-risk infants may delay the onset of the disease, with potential benefit related to maintenance of a state of health during a crucial period of child development.

Although there are many good reasons to recommend prolonged breast-feeding of children, regardless of whether they have a genetic risk for the development of celiac disease, we did not discern a protective effect against celiac disease. Additional studies are needed to establish whether key environmental factors, such as the composition of microbiota, metabolic profile, vaccination schedule, and use of antibiotics, affect the tolerance–immune response equilibrium in these at-risk infants during the first 5 years of life.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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