Risk of Celiac Disease Autoimmunity and Timing of Gluten Introduction in the Diet of Infants at Increased Risk of Disease

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Context While gluten ingestion is responsible for the signs and symptoms of celiac disease, it is not known what factors are associated with initial appearance of the disease.

Objective To examine whether the timing of gluten exposure in the infant diet was associated with the development of celiac disease autoimmunity (CDA).

Design, Setting, and Patients Prospective observational study conducted in Denver, Colo, from 1994-2004 of 1560 children at increased risk for celiac disease or type 1 diabetes, as defined by possession of either HLA-DR3 or DR4 alleles, or having a first-degree relative with type 1 diabetes. The mean follow-up was 4.8 years.

Main Outcome Measure Risk of CDA defined as being positive for tissue transglutaminase (tTG) autoantibody on 2 or more consecutive visits or being positive for tTG once and having a positive small bowel biopsy for celiac disease, by timing of introduction of gluten-containing foods into the diet.

Results Fifty-one children developed CDA. Findings adjusted for HLA-DR3 status indicated that children exposed to foods containing wheat, barley, or rye (gluten-containing foods) in the first 3 months of life (3 [6%] CDA positive vs 40 [3%] CDA negative) had a 5-fold increased risk of CDA compared with children exposed to gluten-containing foods at 4 to 6 months (12 [23%] CDA positive vs 574 [38%] CDA negative) (hazard ratio [HR], 5.17; 95% confidence interval [CI], 1.44-18.57). Children not exposed to gluten until the seventh month or later (36 [71%] CDA positive vs 895 [59%] CDA negative) had a marginally increased risk of CDA compared with those exposed at 4 to 6 months (HR, 1.87; 95% CI, 0.97-3.60). After restricting our case group to only the 25 CDA-positive children who had biopsy-diagnosed celiac disease, initial exposure to wheat, barley, or rye in the first 3 months (3 [12%] CDA positive vs 40 [3%] CDA negative) or in the seventh month or later (19 [76%] CDA positive vs 912 [59%] CDA negative) significantly increased risk of CDA compared with exposure at 4 to 6 months (3 [12%] CDA positive vs 583 [38%] CDA negative) (HR, 22.97; 95% CI, 4.55-115.93; $P=.001$; and HR, 3.98; 95% CI, 1.18-13.46; $P=.04$, respectively).

Conclusion Timing of introduction of gluten into the infant diet is associated with the appearance of CDA in children at increased risk for the disease.

JAMA. 2005;293:2343-2351 www.jama.com

CELIAC DISEASE, ALSO CALLED gluten-sensitive enteropathy, is characterized by chronic inflammation in the small intestine, resulting in villous atrophy and flattening of the mucosa, induced by prolamins (gluten) present in wheat, barley, or rye.[1,2] The classic form of celiac disease typically presents in early childhood with abdominal pain and diarrhea, malabsorption, and nutrient deficiencies. Most patients with celiac disease carry the human leukocyte antigen HLA-DRB1*03 allele (usually associated with HLA-DQ2) or HLA-DRB1*04 (associated with HLA-DQ8).[1,3] These alleles also confer increased risk for type 1 diabetes; thus, individuals with type 1 diabetes and their first-degree relatives have increased risk of celiac disease.[4] However, few genetically susceptible individuals develop ce-

See also p 2410 and Patient Page.

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liac disease, even though virtually all individuals in wheat-consuming populations are exposed to gluten. This suggests that additional factors play a role in disease risk.

Previous case-control studies have reported that children with celiac disease were less likely to have been breastfed or were breastfed for a shorter period of time than children without celiac disease.[5-8] Ivarsson et al[9] found that children with celiac disease were less likely to have been breastfeeding when gluten was introduced into the diet than children without celiac disease. While no effect of the timing of the introduction of gluten into the infant diet on risk of celiac disease has been observed,[5-10] the greater amount of gluten consumed when first introduced increased risk in one[9] but not in another[10] study. These studies suggest that the infant diet may be important in the etiology of celiac disease, but they lack consistency, possibly due to limitations of the case-control study design. Given the known dietary etiology of celiac disease (ie, gluten intake), it is problematic to examine infant diets after celiac disease appears because the participant (or the parents) may be sensitized to the fact that something in the diet "caused" celiac disease and may respond to dietary surveys in a different way than controls, creating bias. A better way to examine this question is to collect the exposure data before the study participants develop disease; however, the low prevalence of celiac disease precludes a longitudinal study in the general population. However, HLA genotype can be used to define a higher-risk cohort, and markers, such as autoantibodies, provide a tool to screen for early presymptomatic disease. The enzyme tissue transglutaminase (tTG) has been identified as the celiac disease autoantigen.[11] The presence of tTG autoantibodies is highly sensitive (0.92-1.00) and specific (0.91-1.00) for celiac disease.[12-16]

The Diabetes Autoimmunity Study in the Young (DAISY) is a prospective study of the natural history and environmental triggers of diabetes and celiac disease autoimmunity (CDA) in genetically predisposed children. Recently we found that first exposure to cereals in the first 3 months of life or in the seventh month or later increased risk of type 1 diabetes autoimmunity in children at risk for diabetes.[17] The objective of our study was to investigate whether there was a similar association between timing of exposure to cereals and subsequent development of celiac disease–associated tTG autoantibodies in children with a genetic predisposition for celiac disease. As in the previous study,[17] we used 4 to 6 months of age as the reference period because the American Academy of Pediatrics recommends the first introduction of solid foods during this time.

METHODS

Study Population

DAISY is investigating the natural history of islet and transglutaminase autoimmunity in infants and children who are at increased risk of developing type 1 diabetes[18] and celiac disease.[19] Increased risk is defined either by having a sibling or parent with type 1 diabetes or having HLA genotypes associated with celiac disease and type 1 diabetes. Newborn children with a sibling or parent with type 1 diabetes were identified from families attending clinics in the Denver metropolitan area, the majority from the Barbara Davis Center for Childhood Diabetes, and recruited regardless of their HLA genotype. Children were also identified at St Joseph's Hospital in Denver, Colo, by screening umbilical cord blood samples for diabetes- and celiac disease-susceptibility alleles in the HLA region. The St Joseph's Hospital newborn population is representative of the general population of the Denver metropolitan area. We excluded families in which parents had difficulties understanding English or whose newborn infant had a severe congenital malformation or disease. Eighty-six percent of the families approached gave informed consent to the genetic screening.

From December 1993 to January 2003, more than 28 100 cord blood samples were screened at Roche Molecular Systems Inc, Alameda, Calif. The details of the newborn screening have been published elsewhere.[18] Children with the following HLA genotypes were invited soon after birth to participate in the DAISY follow-up: DRB1*03, DQB1*0201/DRB1*03, DQB1*0201; DRB1*04,DQB1*0302/DRB1*03, DQB1*0201; DRB1*04, DQB1*0302/DRB1*04,DQB1*0302; and DRB1*04,DQB1*0302/x (where x is neither DRB1*04, DQB1*0302 nor DRB1*03 nor DR2). Two years after their newborn screening, children with the genotype DRB1*03,DQB1*0201/x were also invited to participate in follow-up. This was done to supplement the DAISY cohort with additional children at increased risk for celiac disease. For the remainder of this report, we refer to DRB1*03,DQB1*0201 as DR3. This study was approved by the Colorado Multiple Institutional Review Board. Written informed consent was obtained from the parents of all children.

Infant diet data were collected during telephone or face-to-face interviews at 3, 6, 9, 12, and 15 months of age in those children enrolled soon after birth. At each interview, mothers were asked to report all types of milk, formulas, and foods that the infant consumed over the previous 3 months. If a particular item was introduced for the first time during that 3-month interval, the mother was asked to report the date at first introduction. Breastfeeding initiation and termination were also recorded. For the children enrolled between the ages of 2 and 3 years, the same dietary information was collected retrospectively by questionnaire at enrollment, and in all cases before the appearance of tTG autoantibodies. No dietary advice was given to the families.

Measurement of tTG Autoantibodies

Children followed up from birth had their blood drawn at clinic visits at 9, 15, and 24 months and annually thereafter for the measurement of tTG autoantibodies. Children initially en-

rolled between the ages of 2 and 3 years had their blood drawn at enrollment and annually thereafter. A radioimmunoassay with in vitro transcribed and translated human tTG complementary DNA was used to detect IgA antibodies to tTG in serum samples stored at −20°C, as previously described.[20,21] Briefly, results were expressed as an index, with the cutoff value corresponding to 3 times the highest value for 184 endomysial antibody-negative healthy control participants with a median age of 15.6 years.[20] Samples were tested in duplicate, and all positive samples and 10% of negative samples were confirmed by blinded duplicate testing. In all children, serum total IgA was measured using a nephelometric method, using a cutoff of 3 SDs below the age-adjusted norm or 10 mg/dL. In samples from children found to be IgA deficient, IgG tTG was measured (n=163), and 2 were positive for both IgG tTG and IgA tTG. This definition of tTG positivity has previously been shown to correspond to a 70% to 83% positive predictive value for generally asymptomatic celiac disease without long-term follow-up in this study cohort.[21] Children with a positive tTG result were followed up more frequently, at 3- to 6-month intervals, with repeated testing. All of the positive children had at least 1 negative tTG test prior to their positive tTG test, which means we can determine the interval within which tTG appeared.

Clinical Evaluation and Intestinal Biopsy

After 1 or 2 positive tTG autoantibody results, clinical evaluation and small bowel biopsy were offered. Clinical evaluation included a physical examination and a symptom questionnaire. Intestinal biopsy specimens were obtained initially with a Carey capsule (Wilson-Cook Medical Inc, Winston-Salem, NC [n=4]) and then by upper gastrointestinal endoscopy with 2 to 4 specimens from the descending duodenum. A single pathologist (J.E.H.), blinded to clinical information, assessed the biopsy specimens according to the scoring system described by Marsh[22] (ie, a score of 0 [normal], 1 [infiltrative lesion with increased intraepithelial lymphocytes], 2 [hyperplastic lesion with hyperplastic crypts and increased intraepithelial cells], and 3 [destructive lesion with villous atrophy that may be subtotal, partial or total]). A score of 2 or 3 is considered confirmatory for celiac disease.

Definition of CDA Outcome

The outcome of interest was the time to development of CDA, which was defined as the presence of tTG autoantibodies on 2 consecutive visits or a positive small bowel biopsy after only a single tTG-positive visit. As a secondary, more stringent outcome, we limited our CDA cases to only those children who had a biopsy positive for celiac disease, as defined by a Marsh score of 2 or higher.

Definition of Variables

Gluten exposure was defined as intake of foods containing wheat, barley, or rye, including infant cereals, zwieback, breads, crackers, tortillas, teething biscuits, cookies, cakes, pretzels, and pasta. We examined oats separately because, while oats are not a gluten-containing grain, they are often contaminated by gluten-containing grains during harvesting and milling. We defined rice exposure as intake of foods containing rice, such as infant rice cereal, boiled rice, rice milk, rice cakes, or rice noodles. We defined exposure to cow's milk as intake of any formulas, milk, or foods containing cow's milk, yogurt, cheese, or milk products of any kind. We examined 2 variables related to breastfeeding: (1) duration of breastfeeding (including partial), and (2) whether or not the child was still breastfed when first exposed to gluten. We examined descriptive variables such as sex, maternal education level (categorized as ≤12 or >12 years), and maternal age (in years). We examined race/ethnicity as a potential confounder because of (1) differences in risk of celiac disease by race/ethnicity[23] and (2) differences in infant diet choices by race/ethnicity.[24] Parents reported the race/ethnicity of their child. HLA-DR3 status (HLA-DR3/3, DR3/X vs all other) and family history of type 1 diabetes were examined as potential covariates because they comprised the inclusion criteria for the cohort.

Statistical Analyses

All analyses were performed with SAS version 8 (SAS Institute Inc, Cary, NC). Pearson correlation coefficients were used to examine the correlation between the ages at introduction of cereals and milks into the infant diet. Ongoing recruitment since 1994 and continuing follow-up have resulted in variable lengths of follow-up for the children, producing right-censored data. We began the calculation of follow-up time at birth, and the age of the first positive tTG autoantibody measurement was used to define the time to event. Kaplan-Meier curves were used to describe the risk of CDA by age and in the different exposure groups, and differences in these curves were tested using the Wilcoxon test for equality over strata. Our data are interval-censored because we only know the time of the last negative and first autoantibody-positive blood draw, rather than the actual time of conversion to autoantibody positivity. Therefore, all unadjusted and adjusted hazard ratios (HRs) were estimated using survival analysis (SAS Proc Lifereg) accounting for right and interval censoring.[25] The Weibull distribution was chosen after a comparison of survival models using different distributions found the Weibull distribution to be the better fit. Variables were included in the final model if they were statistically significant (based on the Wald χ^2 P value) or if their inclusion in the model altered the HR of the variable of interest by 10% or more. A P value of $<.05$ was considered statistically significant.

RESULTS

Of the HLA-screened newborns, 55% of families agreed to participate. Of the HLA-screened children recruited at age 2 to 3 years, 67% agreed to partici-

Figure 1. Percentage of the Study Cohort Exposed to Selected Foods by Age

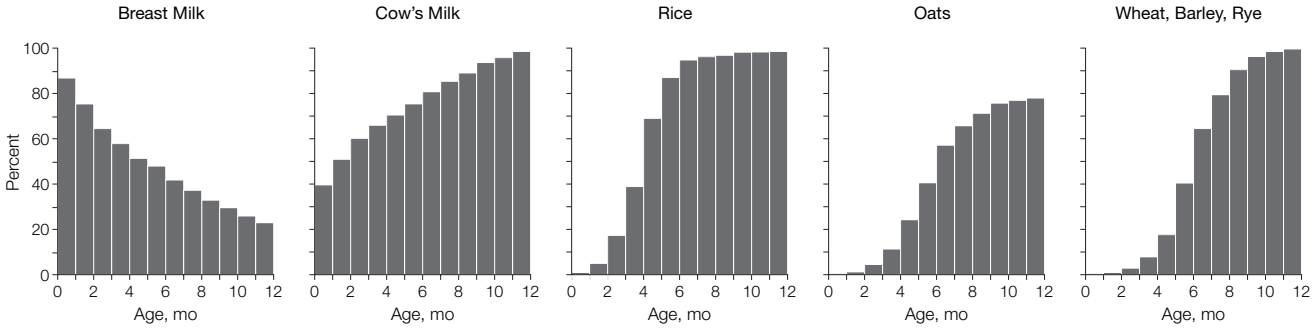

Table 1. Clinical Characteristics of Children With Celiac Disease Autoimmunity

Characteristic	With Biopsy: Positive (n = 25)*	With Biopsy: Negative (n = 9)†	Without Biopsy (n = 17)
HLA-DR3/X or DR3/3 positive, No. (%)	22 (88)	7 (78)	14 (82)
Age at first positive tTG, mean (SD), y	4.6 (1.4)	4.2 (1.4)	5.2 (1.5)
Highest tTG value, mean (range)	1.10 (0.17-2.59)	0.55 (0.09-1.65)	0.81 (0.14-1.50)
TG positive on ≥2 consecutive visits, No. (%)‡	18 (72)	8 (89)	17 (100)
Age at biopsy, mean (SD), y	5.9 (1.5)	5.4 (1.3)	NA
Symptoms in tTG-positive children, No. (%)‡			
None	7 (18)	5 (55)	7 (41)
Diarrhea	3	0	1
Constipation	9	2	1
Gas	2	1	1
Bleeding	2	0	2
Failure to gain weight	8	1	2
Anemia	0	0	1
Short stature	2	1	0
Vomiting	1	0	1
Abdominal pain	6	3	5
Irritability	3	1	2

Abbreviations: NA, not applicable; tTG, tissue transglutaminase.
*The histologic changes as detected by the small bowel biopsy have been described by Marsh[22] as a score of 0 (normal), 1 (infiltrative lesion with increased intraepithelial lymphocytes), 2 (hyperplastic lesion with hyperplastic crypts and increased intraepithelial cells), and 3 (destructive lesion with villous atrophy that can be subtotal, partial, or total). A score of 2 or 3 is considered confirmatory for celiac disease.[15] Four study children had a Marsh score of 2, 20 study children had a Marsh score of 3, and 1 study child had a biopsy done outside the study that was reported as "positive for celiac disease," but no Marsh score was provided.
†One child had a Marsh score of 1.
‡Prior to biopsy in those with biopsy or any time prior to last follow-up in those without biopsy.

pate. We do not have a denominator to calculate the participation rate of those recruited from clinics. We obtained outcome data (tTG autoantibodies) on 84% of children for whom we collected infant diet information. Therefore, the analysis cohort comprised 1560 children, including 1307 children who were followed up from birth (311 [20%] with a family history of type 1 diabetes who were identified at clinics and 996 [64%] identified by newborn HLA screening) and 253 (16%) children followed up from the age of 2 to 3 years. Fifty-one children had CDA, 50 with 2 positive tTG autoantibody measurements and 1 with a single positive measurement and a positive small bowel biopsy. Of these 51 children, 32 completed the study evaluation and biopsy, 2 had a biopsy outside the study, and 17 did not have a biopsy.

The majority of the children (73% [n=1140]) were non-Hispanic white. The remaining 420 children were Hispanic (n=320; 21% of total), biracial (n=50; 3%), African American (n=36; 2%), or other race or missing (n=14; 1%). More than 1 sibling from a family was included in some instances—2 siblings from 143 families and 3 siblings from 10 families. Eighty-seven percent (n=1356) of the cohort was breastfed. Breastfeeding duration was correlated with age at first exposure to rice cereals (r=0.25 [P<.001]) and with oat cereals (r=0.18 [P<.001]), but not with cereals containing wheat, barley, or rye (r=0.03 [P=.31]). Ages at first exposure to rice and oat cereals were correlated with age at first exposure to cereals containing wheat, barley, or rye (r=0.27 [P<.001] and r=0.34 [P<.001], respectively).

FIGURE 1 displays the percentage of children exposed to breast milk, cow's milk, rice, oats, and wheat, barley, or rye at different ages in months. By their 6-month birthday, 87% of the children were eating rice, 40% were eating oats, and 40% were eating wheat, barley, or rye, largely in the form of cereals. At this same age, 48% of the children were still breastfed and 75% had been exposed to cow's milk, largely in the form of infant formula.

TABLE 1 describes the clinical and symptomatic characteristics of the tTG autoantibody–positive children by biopsy status. Children who did not have a biopsy were slightly older than those who did have a biopsy, but were similar to children undergoing biopsy in

terms of HLA-DR3 status, highest tTG autoantibody level, and presence of symptoms. Children with a negative biopsy were similar to those with a positive biopsy in terms of HLA-DR3 status, but were slightly younger, had lower tTG autoantibody levels, and were less likely to report symptoms, likely reflecting an earlier course of the disease.

Infant Diet Exposures and Risk of CDA

The mean (SD) age at first positive tTG autoantibody test for the 51 CDA-positive children was 4.7 (1.5) years, and the mean (SD) age at the last follow-up for the 1509 CDA-negative children in the cohort was 4.8 (2.9) years (TABLE 2). Of the CDA-positive children, 3 (6%) were exposed to wheat, barley, or rye at 1 to 3 months, 12 (23%) at 4 to 6 months, and 36 (71%) at 7 months or later, vs 40 (3%), 574 (38%), and 895 (59%) of CDA-negative children. Kaplan-Meier curves displaying the proportion of children becoming CDA positive by time period of exposure to wheat, barley, or rye were significantly different from each other ($P=.04$) (FIGURE 2). All of the variables listed in Table 2 were considered for inclusion in the multivariate survival analysis model, and only HLA-DR3 status met the statistical criteria for inclusion. Adjusting for HLA-DR3 status, children exposed to wheat, barley, or rye in the first 3 months of life had a 5-fold increased hazard of CDA compared with those who were exposed at 4 to 6 months (TABLE 3). Children not exposed to wheat, barley, or rye until their seventh month or later were at a slightly increased hazard of CDA compared with those who were exposed in the 4- to 6-month period, which was only marginally significant. Further adjustment for the other cereal variables demonstrated that the association between CDA and exposure to wheat, barley, or rye was independent of the age at first exposure to rice and to oats. Of the 25 children with biopsy-confirmed CDA-positive status, 3 (12%) were exposed to wheat, barley, or rye at 1 to 3 months, 3 (12%) at 4 to 6 months, and 19 (76%) at 7 months or later vs 40 (3%), 583 (38%), and 912 (59%) of unaffected children, respectively. Initial exposure to wheat, barley, or rye in the first 3 months or in the seventh month or later significantly increased risk of biopsy-confirmed CDA compared with exposure at 4 to 6 months (Table 3).

To examine whether the inclusion of the 253 children with retrospective di-

Table 2. Descriptive Characteristics of Cohort by Celiac Disease Autoimmunity (CDA) Status*

Characteristic	CDA Positive (n = 51)	CDA Negative (n = 1509)	Unadjusted Hazard Ratio (95% CI)
Age, mean (SD), y†	4.7 (1.5)	4.8 (2.9)	
Race/ethnic group			
Non-Hispanic white	40 (78)	1100 (73)	1.07 (0.55-2.11)
Other	11 (22)	404 (27)	1.00
Sex			
Male	25 (49)	793 (53)	0.85 (0.49-1.47)
Female	26 (51)	716 (47)	1.00
HLA genotype			
DR3/X or DR3/3	43 (84)	734 (49)	5.98 (2.81-12.73)
DRX/X	8 (16)	775 (51)	1.00
First-degree relative with type 1 diabetes			
Yes	17 (33)	541 (36)	1.01 (0.57-1.82)
No	34 (67)	968 (64)	1.00
First-degree relative with celiac disease			
Yes	2 (4)	11 (0.7)	6.60 (1.6-27.2)
No	49 (96)	1498 (99)	1.00
Maternal education, y			
≤12	7 (14)	346 (23)	0.57 (0.26-1.26)
>12	44 (86)	1127 (77)	1.00
Maternal age at birth, mean (SD), y	31 (5.7)	30 (5.7)	1.03 (0.98-1.08)‡
z Score of weight for age at first visit, mean (SD)§	–0.42 (1.14)	–0.35 (1.07)	0.96 (0.72-1.29)‡
Birth weight, mean (SD), lb	7.3 (1.3)	7.4 (1.3)	0.96 (0.77-1.19)‡
Age initially exposed to rice, mo			
1-3	8 (16)	262 (17)	0.83 (0.38-1.78)
4-6	35 (69)	1054 (70)	1.00
≥7	8 (16)	193 (13)	1.14 (0.53-2.46)
Age initially exposed to oats, mo			
1-3	3 (6)	67 (4)	1.57 (0.44-5.56)
4-6	12 (23)	548 (36)	1.00
≥7	36 (71)	894 (60)	1.48 (0.77-2.84)
Age initially exposed to wheat, barley, or rye, mo			
1-3	3 (6)	40 (3)	2.94 (0.83-10.4)
4-6	12 (23)	574 (38)	1.00
≥7	36 (71)	895 (59)	1.78 (0.92-3.42)
Age initially exposed to cow's milk, mo‖			
1-3	28 (55)	908 (60)	1.37 (0.57-3.31)
4-6	6 (12)	235 (16)	1.00
≥7	17 (33)	366 (24)	1.74 (0.69-4.42)
Breastfeeding duration, mean (SD) [median], mo	8.3 (8.8) [5]	6.7 (6.8) [5]	1.02 (0.99-1.05)‡
Breastfed when first exposed to wheat, barley, or rye			
Yes	25 (49)	660 (44)	1.32 (0.76-2.28)
No	26 (51)	849 (56)	1.00

*Data are presented as number (percentage) unless otherwise indicated. Data on ethnicity were missing for 5 children; data on maternal education were missing for 36 children.
†For CDA-positive children, "age" is age at visit when they first tested positive for autoantibodies. For CDA-negative children, "age" is age at last visit.
‡95% Confidence interval (CI) calculated for a 1-unit difference (eg, month, year, pound).
§First visit occurred between 8 and 16 months of age (no weight measurement at these ages for 10 CDA-positive and 239 CDA-negative children).
‖Includes milk-based infant formulas, cow's milk, and all milk-containing foods.

etary data may have affected our findings, we limited our cohort to only those children for whom data were collected prospectively (n=1307 children, of whom 43 were CDA positive). The adjusted HRs for exposure to wheat, barley, or rye at 0 to 3 months and 7 months or later (compared with 4-6 months) were 7.49 (95% confidence interval [CI], 1.53-36.60) and 2.96 (95% CI, 1.31-6.67) respectively, suggesting an increased risk of CDA for both early and late initial exposure to wheat, barley, and rye. This subanalysis suggests that inclusion of children with retrospective data did not negatively affect our results.

To determine whether inclusion of multiple siblings per family affected our results, we randomly selected 1 sibling per family and reran our analyses. The adjusted HRs for exposure to gluten at 0 to 3 months and 7 months or later (compared with 4-6 months) were 5.65 (95% CI, 1.19-26.78) and 2.39 (95% CI, 1.09-5.25) respectively, suggesting an increased risk of CDA for both early and late initial exposure to wheat, barley, and rye. This subanalysis suggests that inclusion of multiple siblings did not affect our results.

We then limited our analyses to the children at highest risk for celiac disease, those who have at least 1 HLA-DR3 allele (n=777), in whom 43 cases of CDA developed. Kaplan-Meier curves showing the proportion of children becoming CDA positive by month of exposure to wheat, barley, or rye in HLA-DR3–positive children were significantly different from each other ($P=.004$) (Figure 2). Of the 14 DR3-positive children who were exposed to wheat, barley, or rye in the first 3 months of life, the risk of CDA was 40% by 5.5 years of age. The HRs (95% CIs) for CDA in children initially exposed to wheat, barley, or rye in the first 3 months or not until the seventh month or later were 7.28 (2.02-26.25) and 1.68 (0.84-3.36), respectively, compared with children exposed in the 4- to 6-month period, suggesting an increased risk of CDA with early exposure to wheat, barley, and rye in HLA-

Figure 2. Celiac Disease Autoimmunity (CDA) Risk by Month of Exposure to Foods Containing Wheat, Barley, or Rye in Entire Cohort and in Children With Positive HLA-DR3 Status

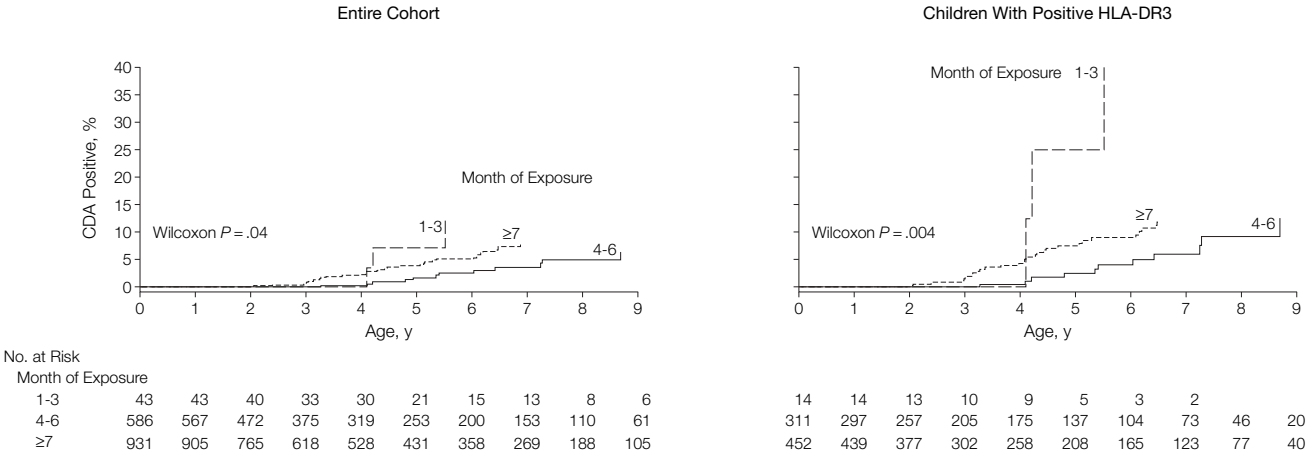

Table 3. Risk of Celiac Disease Autoimmunity (CDA) by Month of Exposure to Foods in the Infant Diet in 1560 Children*

	1-3 mo		4-6 mo	≥7 mo	
	Hazard Ratio (95% CI)	*P* Value†	Hazard Ratio	Hazard Ratio (95% CI)	*P* Value†
Wheat, barley, rye					
Risk of CDA (n = 51)	5.17 (1.44-18.57)	.02‡	1.00	1.87 (0.97-3.60)	.07‡
Risk of CDA in children with positive biopsy (n = 25)§	22.97 (4.55-115.93)	.001	1.00	3.98 (1.18-13.46)	.04
Oats					
Risk of CDA (n = 51)	2.02 (0.57-7.17)	.28	1.00	1.54 (0.80-2.96)	.21
Risk of CDA in children with positive biopsy (n = 25)§	4.25 (1.06-17.06)	.05	1.00	1.37 (0.53-3.51)	.52
Rice					
Risk of CDA (n = 51)	0.86 (0.40-1.86)	.71	1.00	1.25 (0.58-2.69)	.57
Risk of CDA in children with positive biopsy (n = 25)§	0.90 (0.30-2.68)	.85	1.00	1.24 (0.42-3.70)	.69

Abbreviation: CI, confidence interval.
*Separate models were run for each infant food, adjusted for positive HLA-DR3 status. The referent category was 4 to 6 months.
†*P* Value from the Wald χ^2 test, with 4-6 months as the reference category.
‡Further adjustment of the wheat, barley, or rye model for age at initial exposure to oats and to rice resulted in adjusted hazard ratio (95% CI) for exposure to wheat, barley, and rye at 0 to 3 months, and 7 months or older (compared with 4-6 months) of 7.08 (1.24-40.56) and 1.70 (0.87-3.31), respectively.
§Positive biopsy defined as Marsh score of 2 or higher (see the "Methods" section).

DR3–positive children. It was not possible to test for an interaction between HLA-DR3 and timing of exposure to gluten because there were no CDA cases that were DR3 negative and exposed in the first 3 months of life.

COMMENT

We found an association between timing of initial exposure to wheat, barley, and rye and the development of CDA. Wheat, barley, and rye are closely related grasses that contain prolamins, or storage proteins, that induce the autoimmune process in patients with celiac disease.[26] Treatment for celiac disease is a gluten-free diet, which essentially eliminates foods containing wheat, barley, and rye from the diet, as well as gluten used as an additive. While we combined wheat, barley, and rye into a single exposure category, it should be noted that wheat and barley made up the great majority of exposures in infants because of the availability of commercial cereals made from wheat and/or barley. Individuals with celiac disease are also advised to avoid oats because they may be contaminated with wheat (and thus gliadin) through the harvesting and milling process, even though oats themselves are well-tolerated by most individuals with celiac disease.[27-31] We found no association between the development of CDA and the timing of introduction of oats or of rice, suggesting that the association is antigen-specific.

We chose our reference group to be those exposed at 4 to 6 months because pediatricians in the United States generally recommend the introduction of solid foods, particularly cereals, between 4 and 6 months, although there is no official American Academy of Pediatrics practice guideline regarding this.[32] Our data suggest that introducing foods containing gluten in the first 3 months of life increases a child's risk of CDA. Interestingly, waiting until the seventh month or later to first introduce foods containing gluten marginally increases the risk for CDA compared with introducing gluten in the 4- to 6-month period.

We were not able to confirm celiac disease in all tTG autoantibody–positive children via small bowel biopsy. To examine whether our findings might be generalizable to celiac disease itself, we restricted our CDA cases to those 25 children who were subsequently diagnosed with celiac disease based on small bowel biopsy and found that initial exposure to wheat, barley, or rye in the first 3 months or in the seventh month or later significantly increased risk. This suggests a window of exposure to gluten outside of which one may increase CDA risk in susceptible children.

Gliadin deamidation by tTG has been demonstrated to enhance the recognition of gliadin peptides by T cells and this might initiate the cascade of autoimmune reactions leading to celiac disease.[33,34] For this to happen, gliadin has to cross the intestinal epithelial barrier so that it can be recognized by antigen-presenting cells. While the intestinal barrier functions as the major organ of defense against foreign antigens, toxins, and macromolecules entering the host via the oral route, at very young ages this barrier may not be as complete as at older ages, thus allowing gliadin to pass even with small amounts of intake.

The reason why late gluten exposure is also associated with CDA is less clear. When wheat is introduced to an older child, it tends to be introduced in greater amounts, thus increasing the amount of gliadin available to cross the gut. Even if a small proportion of the available gliadin crosses the gut, it may be sufficient to initiate the cascade. Ivarsson et al[9] found that children with celiac disease were exposed to a larger amount of gluten at first exposure than children without celiac disease, and that this amount at initial exposure increased the later in life gluten was introduced. In our study, infants first exposed to cereals at or after the seventh month were more likely to have been given 1 or more servings per day in the first month of exposure compared with children who were first exposed before 4 months (52% vs 31%, respectively), suggesting that the frequency of exposure at initial introduction increased with age. The recent finding that gliadin may actually activate a zonulin-dependent enterocyte intracellular signaling pathway leading to increased intestinal permeability[33] suggests a cycle where dietary intake of gliadin would lead to increasing intestinal permeability via the activation of zonulin by gliadin, which would in turn lead to greater and greater exposures to the body with continued gliadin intake.

Although all children in our cohort were exposed to gluten by 12 months of age, the first positive tTG autoantibody result did not occur until 2 years of age, with a mean age at conversion of 4.7 years, showing a delay in the appearance of tTG autoantibodies. This might be due to less sensitive serology in the youngest age group. We were not aware of data on human tTG autoantibodies in children younger than 2 years, so we reviewed laboratory data on diabetic individuals at the Barbara Davis Center for Childhood Diabetes. Tissue transglutaminase autoantibodies were present at diabetes onset in 5.7% of children younger than 2 years and in 7.8% of older children, suggesting that tTG autoantibodies are detectable at young ages with our assay (G.S.E., unpublished data, 2005). Therefore, the delayed appearance of tTG autoantibodies is consistent with an immature developing immune system slowly responding to exposure to gluten and fueled by continued gluten exposure and perhaps other exposures as well. This recognition of a delay in appearance of autoantibodies is reflected in a recent recommendation to screen high-risk asymptomatic children for tTG autoantibodies after the age of 3 years, provided they have been receiving an adequate gluten-containing diet for at least 1 year.[35]

Because of the similarities between the epidemiology of celiac disease and type 1 diabetes and the coexistence of the 2 diseases in the same individuals or families, gluten exposure is also a candidate risk factor for type 1 diabetes.[36] We recently published findings from the DAISY cohort showing that children ini-

tially exposed to cereals in the first 3 months of life and those who were not exposed until the seventh month or later had an increased risk of islet autoimmunity compared with those who were exposed at 4 to 6 months.[17] However, the association with islet autoimmunity was not specific to gluten-containing foods. Due to differences in inclusion and exclusion criteria, the cohort from our previous report and that of the current study are not entirely the same, although they are drawn from the same DAISY population. Only 3 children were positive for both islet autoimmunity and CDA, and thus were counted as cases in both analyses, so it is unlikely that one form of autoimmunity is driving the association of the other. Ziegler et al[37] found that exposure to gluten in the first 3 months of life increased risk of islet autoimmunity 5-fold in offspring of type 1 diabetic individuals. The investigators also reported an inverse relationship between age at gluten exposure and tTG autoantibody risk, but this was not significant, perhaps due to the small number of tTG autoantibody cases. These prospective studies of 2 childhood autoimmune diseases suggest that this period during infancy is important for the development of the immune system and potentially in determining the difference between tolerance and sensitization to specific food antigens.

We did not observe the protective effect of breastfeeding reported in previous retrospective case-control studies.[5-9] Aside from the methodological differences between retrospective and prospective studies regarding selection and recall bias, this lack of replication may be related to inherent infant diet differences across studies. The studies showing a protective association of breastfeeding were done in Europe where infant diet practices may be different compared with the United States. In our population, the first exposure to wheat, barley, or rye was primarily in the form of infant cereals, which are not replacements for breast milk and in fact are not correlated with breastfeeding duration. In other countries, the primary exposure to gluten may be more correlated with breastfeeding duration, for example, the flour-based follow-up infant formula used in Sweden. The previous studies may not have been able to disentangle breastfeeding from gluten introduction due to imprecision in their retrospective data.

In summary, timing of gluten introduction into the infant diet is associated with risk of CDA. We note that our outcome was not celiac disease per se; however, our analysis of CDA cases with biopsy-confirmed celiac disease suggests that this association may exist with clinical disease as well. Given that our study population was selected for specific genetic and family history characteristics, our findings are generalizable only to children at increased risk for celiac disease. We cannot exclude the possibility that earlier exposure to gluten simply leads to earlier appearance of CDA and that all exposed at-risk children will eventually develop CDA. Long-term follow-up of this cohort may be necessary to address this question. Given the small number of CDA-positive children and wide CIs, we recommend that these results be confirmed in other prospective cohorts of children at risk for celiac disease before any interventions are implemented. Additional studies may shed light on the importance of quantity of exposure and whether the risk is related to exposure to antigens or to other components of cereals. Our results support continuing current US feeding recommendations for introduction of cereal in infants at 4 to 6 months.

ADDENDUM

The American Academy of Pediatrics recently published a policy statement recommending exclusive breastfeeding for the first 6 months of life, with the gradual introduction of complementary foods (eg, cereals, etc) beginning around 6 months of age. The Academy acknowledges that "unique needs or feeding behaviors of individual infants [could] indicate a need for introduction of complementary foods as early as 4 months of age. . . ."[38]

Author Contributions: Drs Norris and Rewers (principal investigator) had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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Acquisition of data: Norris, Hoffenberg, Taki, Miao, Haas, Emery, Erlich, Eisenbarth, Rewers.
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Administrative, technical, or material support: Norris, Emery, Erlich, Rewers.
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Study coordinator: Taki.
Financial Disclosures: None reported.
Funding/Support: This research was supported by National Institutes of Health grants R01-DK32493, DK50979, DK32083, DK49654, Autoimmune Prevention Center AI 50864, Diabetes Endocrine Research Center, Clinical Investigation & Bioinformatics Core P30 DK 57516, and the General Clinical Research Centers Program, National Center for Research Resources M01RR00069.
Role of the Sponsors: The funding sources did not participate in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.
Acknowledgment: We would like to acknowledge the dedicated and talented staff of the DAISY study for their clinical, data, and laboratory support. We are indebted to all of the children and their families who generously volunteered their time and knowledge.

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