The role of gluten consumption at an early age in celiac disease development: a further analysis of the prospective PreventCD cohort study[1,2]

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ABSTRACT

Background: We previously found that the introduction of small quantities of gluten at 4–6 mo of age did not reduce the risk of celiac disease (CD) in a group of high-risk children. However, the consumption of high amounts of gluten early in life has been suggested to increase CD risk.

Objective: The aim of this study was to evaluate this hypothesis by using data from the previous study of the PreventCD trial (www.preventcd.com).

Design: Gluten intake was prospectively quantified by using specific food records between 11 and 36 mo of age in 715 children positive for the human leukocyte antigen (*HLA*)–*DQ2* and/or *HLA-DQ8* from 5 European countries. According to the PreventCD protocol, infants received 100 mg immunologically active gluten/d or placebo from 4 to 6 mo of age, with a stepwise and fixed gluten increase until age 10 mo and unrestricted intake thereafter. The primary outcome of the present study was the impact of the amount of gluten consumed from age 10 mo onward on CD development.

Results: Mean daily gluten intakes from 10 mo onward were significantly different between countries for children at all ages ($P < 0.001$) but not between children who developed CD and those who did not within the same country ($P > 0.05$). The variables country, sex, intervention group, and gluten consumption pattern did not show significant associations with CD development risk (HRs not significant). In addition, the interaction between *HLA* risk group and gluten consumption pattern showed no significant risk on CD development, except for the DQ2.2/DQ7 haplotype (HR: 5.81; 95% CI: 1.18, 28.74; $P = 0.031$).

Conclusions: Gluten consumption patterns as well as the amount of gluten consumed at 11–36 mo of age do not influence CD development for most related *HLA* genotypes in children with a genetic risk. This study reports the gluten consumption pattern in children at risk of CD from different European countries. This trial was registered at www.controlled-trials.com as ISRCTN74582487. *Am J Clin Nutr* 2017;105:890–6.

Keywords: celiac disease, children, gluten, *HLA*, European cohort

INTRODUCTION

Celiac disease (CD)[16], an immune-mediated systemic disorder triggered by the consumption of gluten and related prolamines, is strongly associated with the human leukocyte antigen (*HLA*)-*DQ2* and *HLA-DQ8* genes. Because ∼30% of the white population carries *HLA-DQ2* but only 1% will develop the disease (1), many studies have focused on environmental triggers that could additionally increase the risk of disease, especially early-feeding practices (2–6). For years, gluten introduction between the ages of 4 and 6 mo was considered a window of opportunity to either prevent or delay the development of CD (7, 8). However, a 2015 systematic review with meta-analysis, including data from current observational and randomized controlled trials (9–13), concluded that the age

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[2] Supplemental Methods, Supplemental Figures 1–3, and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[16] Abbreviations used: CD, celiac disease; FR, food record; *HLA*, human leukocyte antigen; MDGI, mean daily gluten intake; TG2A, tissue transglutaminase type 2 antibody.

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of gluten introduction is not related to the risk of developing CD. With regard to the amount of gluten to be consumed at weaning, data from 2 observational studies and 2 prospective nested case-control studies indicated that the consumption of high amounts of gluten at weaning and during the first 2 y of life increases the risk of developing CD (5, 14–16). However, in the evaluation of gluten intake, different methodologies with different levels of accuracy were used in the referred studies, limiting the possibility to draw any firm conclusions. Obtaining precise data on gluten intake is extremely challenging because of differences in dietary habits among countries but also because of composition variability for similar products and even for the same brands. Therefore, specific, well-designed prospective studies conducted by specialized dietitians are needed to obtain valid information.

Accordingly, we prospectively recorded and calculated the gluten consumption of participants of the European PreventCD birth cohort from the first gluten introduction at ≤36 mo of age, with continued follow-up until 6 y of age. Our aim was to complete the analysis of the previously reported PreventCD study with regard to whether the quantity or pattern of gluten consumption is an independent risk factor for developing CD during childhood.

METHODS

The PreventCD cohort

Infants from 8 European countries (n = 944) with ≥1 first-degree relative with CD and with a high-risk *HLA* were randomly assigned to receive either 100 mg immunologically active gluten/d (gluten group) or 2 g lactose/d (placebo group) at 4 mo of age for 8 wk (months 4–6) (12, 17). After the intervention, all of the parents were thoroughly instructed to progressively increase gluten intake by introducing specific foods containing ascertained gluten amounts with the use of regular products and standardized recommendations: at age 6 mo, 250 mg gluten was recommended; at 7 mo, 500 mg was recommended; at 8 mo, 1000 mg was recommended; and at 9 mo, 1500 mg gluten was recommended. From 10 mo onward, unrestricted gluten consumption was allowed according to national dietary habits, which was quantified at 11, 12, 14, 16, 18, 20, 22, 24, 28, 30, 34, and 36 mo of age.

All of the children were periodically monitored for serum antigliadin and tissue transglutaminase type 2 antibody (TG2A), total IgA in serum, and for symptoms of CD. The diagnosis of CD was confirmed according to the criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (18, 19) (**Supplemental Methods**).

The recruitment of the PreventCD cohort started on 1 January 2007. However, because the PreventCD cohort is still being followed up, for the purposes of this study monitoring and data collection were closed on 30 April 2015 when all children of the cohort had reached 5 y of age.

Study design

The primary outcome of this analysis was to determine the impact of the amount and pattern of gluten consumed on the development of CD. We performed a prospective assessment of mean daily gluten intake (MDGI) in grams per day at 11, 12, 14, 16, 18, 20, 22, 24, 28, 30, 34, and 36 mo of age by using specific food records (FRs) adapted to local dietary habits, with information collected from a 7-d period (20, 21). The FRs and the gluten content of the consumed products were assessed by specialized dietitians in each country. We considered as gluten-containing foods those that included wheat, barley, or rye. Oats consumption at these ages was negligible. The gluten amount was calculated by multiplying the grams of gluten containing vegetable proteins of each reported product by 0.8 according to a generally accepted method (16, 20–22). Different official information sources were checked to determine the exact amount of gluten-containing proteins in each foodstuff and per brand (Supplemental Methods).

Statistical analysis

Data were summarized by their means, SDs in the case of continuous variables, and absolute and relative frequencies in the case of categorical variables. The means of daily gluten intake among countries, as well as the means in CD and non-CD cases for each country, were compared by using t tests and considered

TABLE 1
Characteristics of the study population[1]

Country	n[2]	Intervention group (gluten/placebo),[3] n/n	Sex (M/F), n/n	*HLA* risk group,[4] n children (CD cases)					CD cases,[5] n
				Group 1	Group 2	Group 3	Group 4	Group 5	
Spain	225 (202)	116/109	125/100	51 (13)	18 (2)	98 (9)	11 (0)	47 (0)	24
Germany	100 (95)	48/52	59/41	13 (4)	6 (0)	48 (6)	7 (0)	26 (1)	11
Netherlands	127 (113)	63/64	63/64	16 (2)	8 (3)	78 (10)	4 (1)	21 (2)	18
Hungary	135 (83)	68/67	65/70	21 (8)	11 (3)	69 (11)	7 (0)	27 (2)	24
Italy	128 (104)	62/66	64/64	12 (5)	28 (3)	45 (7)	3 (1)	40 (2)	18
Total	715 (597)	357/358	376/339	113 (32)	71 (11)	338 (43)	32 (2)	161 (7)	95

[1] CD, celiac disease; *HLA*, human leukocyte antigen.
[2] Values represent the total number of children included in the study; the number of children aged 6 y at the time of the study are shown in parentheses.
[3] Number of children who received gluten or placebo from age 4 to 6 mo.
[4] *HLA* group 1 included DQ2.5/DQ2.5 and DQ2.5/DQ2.2; group 2 included DQ2.2/DQ7; group 3 included DQ2.5/DQ7, DQ2.5/DQ8, and DQ2.5/other; group 4 included DQ2.2/DQ2.2, DQ2.2/DQ8, and DQ8/DQ8; and group 5 included DQ2.2/other, DQ8/DQ7, and DQ8/other. Values represent the total number of children included in different groups of *HLA* and the number of children who developed CD within each specific *HLA* risk group.
[5] Additional information on CD cases is provided in Supplemental Table 1.

significant when $P < 0.05$. Data on gluten consumption in children with CD were collected only up to the time of diagnosis; thereafter, these individuals and their dietary intake were excluded from the statistical analysis.

A piecewise linear mixed model was adjusted to establish gluten consumption patterns with the data obtained from the means of daily gluten intake of each country. The gluten consumption pattern was adjusted and defined by this model by including the following variables as covariates: monthly mean gluten increase in MDGI between 11 and 18 mo of age (i.e., initial slope), MDGI at 18 mo, and monthly mean gluten increase in MDGI between months 18 and 36 (i.e., final slope).

We conducted a univariate Cox proportional hazards regression for each individual predictor followed by a multivariable adjusted hazards regression model to assess the hazard risk of each independent variable on the probability of developing CD. The variables included in the statistical model were as follows: country; sex; intervention group; gluten consumption pattern, which included the amount of gluten intake; *HLA* risk group; and the interaction between *HLA* risk group and gluten consumption pattern. Moreover, the same model with the exclusion of the variable country was adjusted to assess the country effect by using the likelihood ratio test.

P values for the mixed models were estimated by using Satterthwaite's approximation to the df and considered significant when $P < 0.05$. All of the statistical analyses were conducted and graphs created with the use of R software (version 3.1.2).

Ethics

This study is within the PreventCD study, which was approved by the local medical ethical committees of the participating centers and complies with the Good Clinical Practices regulations. The study protocol of PreventCD has been previously published (17). This trial was registered at www.controlled-trials.com as ISRCTN74582487.

RESULTS

Of 944 children recruited in the PreventCD study, our study included only those children with detailed data available on gluten consumption. Thus, we obtained 6750 FRs pertaining to 715 children from 5 countries (Germany, Hungary, Italy, Spain, and Netherlands; **Supplemental Figure 1**). At the time of the analysis, 597 (83.5%) of the children were >6 y of age. Characteristics of the study population are shown in **Table 1** and **Supplemental Table 1**.

Gluten intake in the study population

The MDGI and gluten consumption patterns from month 11 onward are presented in **Table 2** and **Supplemental Figure 2**. According to the PreventCD protocol, all of the children had the same fixed gluten consumption between the ages of 6 and 10 mo. However, from 11 mo onward, significant differences in MDGI were observed among countries at different ages ($P < 0.001$), with the exception of The Netherlands and Hungary at age 11 mo ($P = 0.85$). Dutch and Hungarian children consumed 4-fold more gluten compared to the intake at 10 mo of age (from 1.5 to 5.2 g/d). In Spain, MDGI was significantly lower at any age ($P < 0.001$) compared with the other countries.

TABLE 2
MDGIs calculated from the food records in the assessed months and gluten consumption pattern per country[1]

	Spain (n = 225)		Germany (n = 100)		Netherlands (n = 127)		Hungary (n = 135)		Italy (n = 128)	
Age, mo	MDGI (SD)	Estimate (95% CI)	MDGI (SD)	Estimate (95% CI)	MDGI (SD)	Estimate (95% CI)	MDGI (SD)	Estimate (95% CI)	MDGI (SD)	Estimate (95% CI)
11	2.55 (1.27)	0.21[2] (0.15, 0.26)	3.72 (1.8)	0.38[2] (0.26, 0.50)	5.27 (2.15)	0.33[2] (0.23, 0.43)	5.20 (3.39)	0.64[2] (0.54, 0.74)	4.85 (2.16)	0.60[2] (0.50, 0.70)
12	3.14 (1.49)		4.31 (2.11)		6.36 (2.53)		7.07 (3.91)		5.43 (2.92)	
14	3.45 (1.43)		NA		7.68 (2.49)		7.56 (4.19)		7.93 (2.99)	
16	3.87 (1.73)		NA		7.69 (2.42)		9.27 (4.05)		5.80 (3.35)	
18	4.01 (1.72)	4.25[3] (4.15, 4.47)	6.55 (2.68)	6.51[3] (6.18, 6.89)	8.04 (2.54)	8.17[3] (7.95, 8.35)	10.92 (4.25)	10.27[3] (10.08, 10.45)	8.81 (3.9)	8.87[3] (8.43, 8.99)
20	4.20 (1.94)	0[4] (−0.01, 0.03)	NA	0.06[4] (0.01, 0.11)	8.52 (2.44)	0.04[4] (0, 0.08)	9.35 (4.36)	0.03[4] (−0.01, 0.07)	9.76 (4.01)	0.20[4] (0.15, 0.25)
22	4.15 (1.98)		NA		8.30 (2.69)		10.39 (3.96)		8.93 (3.40)	
24	4.37 (1.87)		6.86 (3.02)		8.09 (2.73)		11.25 (4.01)		10.78 (3.94)	
28	4.20 (1.84)		NA		8.60 (2.69)		10.01 (3.55)		11.82 (3.66)	
30	4.15 (1.77)		6.96 (2.87)		8.77 (2.78)		10.61 (3.46)		12.37 (3.96)	
34	4.46 (1.92)		NA		8.85 (2.50)		10.96 (3.38)		11.51 (3.73)	
36	4.39 (1.85)		7.8 (3.94)		9.19 (2.92)		11.51 (3.33)		12.10 (3.22)	

[1] Values of gluten consumption pattern were estimated by using a piecewise linear mixed model from MDGI data. MDGI, mean daily gluten intake in grams per day obtained from the food records of children; NA, data not available.
[2] Increase in gluten intake in grams per month in the initial slope (age 11–18 mo).
[3] Estimated MDGI at age 18 mo.
[4] Increase in gluten intake in grams per month in the final slope (age 18–36 mo).

Gluten consumption patterns were similar in all of the countries but not the amount of gluten consumed. Independently of the amount consumed, we observed a marked monthly increase in gluten intake between 11 and 18 mo of age (defined as the initial slope) in all countries, but a milder increase between 18 and 36 mo of age (defined as the final slope), with the exception of Italy where the consumption of gluten in this period continued to increase sharply (Table 2, Supplemental Figure 2). This means that when unrestricted consumption was allowed, gluten was suddenly introduced at ≤18 mo of age, resulting in a sharp initial slope. Thereafter, consumption increased slowly. Spanish children showed the mildest initial slope, the lowest estimation of MDGI at 18 mo, and practically no increase in gluten intake from 18 to 36 mo (estimated as 0). Hungarian children showed the most striking initial slope and the highest MDGI estimated at 18 mo of age. The highest final slope corresponded to Italian children, with significant differences ($P < 0.001$) compared with the other countries. Altogether, there were significant differences in the initial slope (monthly increase in MDGI intake) varying from 0.21 to 0.64 g/mo; there were also significant differences in MDGI at 18 mo (from 4.25 to 10.27 g/d) among all countries ($P < 0.001$; Table 2).

From 11 to 36 mo of age, gluten consumption at different ages of those children who received gluten during the initial intervention period (4–6 mo of age) was similar to that of children who received a placebo. The overall MDGI in the placebo group was 0.28 g lower than in the gluten group (95% CI: −0.56, 0.01; $P = 0.06$). Boys and girls consumed similar amounts of gluten; the overall effect of male sex was 0.27 g (95% CI: −0.01, 0.56 g) at any age and in all of the countries ($P = 0.06$).

Comparison of gluten intake in the CD and non-CD cases

The MDGI at different ages and in all of the countries was similar among the children who developed CD and those who did not develop CD ($P > 0.05$; **Figure 1**, **Supplemental Table 2**).

Risk of CD development

Results from the Cox proportional hazards multivariable regression model are presented in **Table 3**. The analysis performed to analyze the hazard risks of each independent variable in CD development showed that the variables of country, sex, and intervention group did not have a significant association with CD development risk (P values of HRs = 0.40, 0.22, and 0.59, respectively). In addition, the variable of gluten consumption pattern, which included the amount of gluten consumed, did not

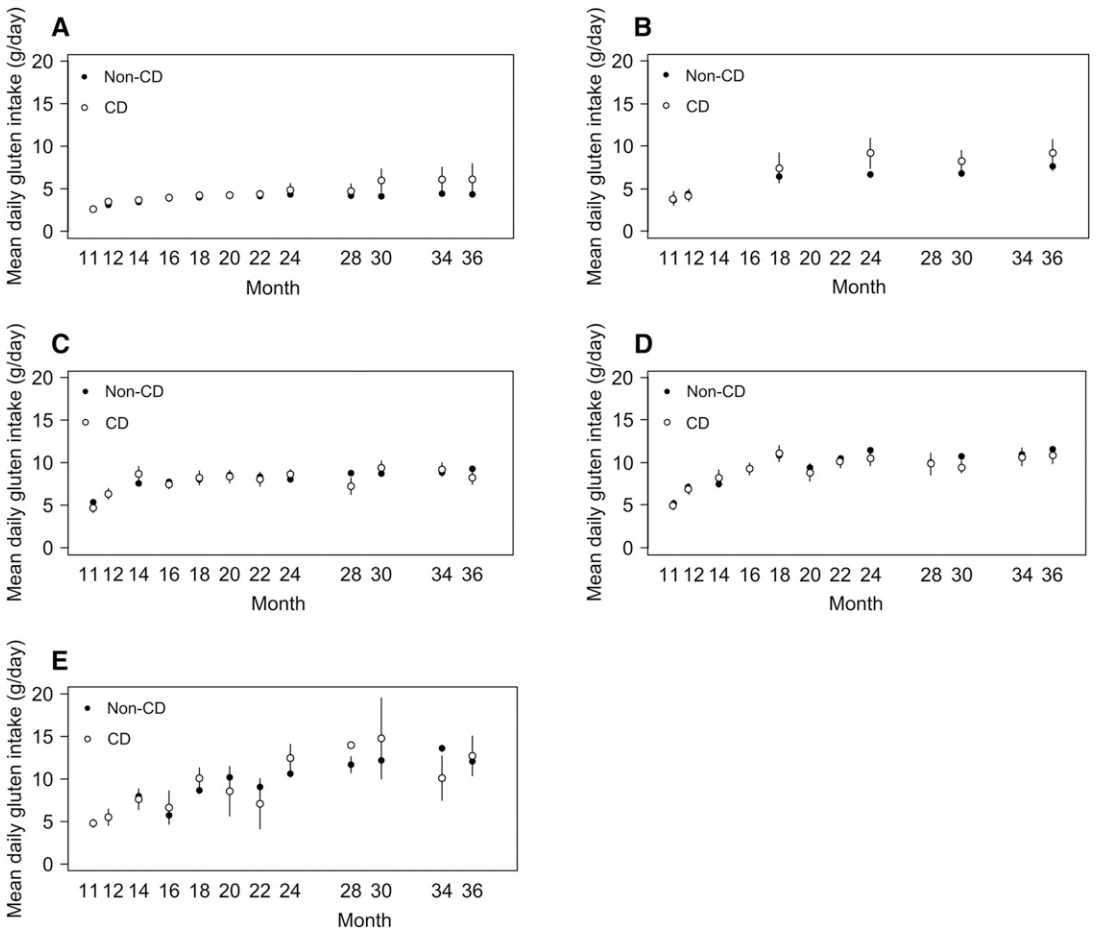

FIGURE 1 Mean daily gluten intakes and SDs (vertical lines) at different ages in CD and non-CD cases by country for Spain (Non-CD = 201, CD = 24) (A), Germany (Non-CD = 89, CD = 11) (B), Netherlands (Non-CD = 109, CD = 18) (C), Hungary (Non-CD = 111, CD = 24) (D), and Italy (Non-CD = 110, CD = 18) (E). The different means were compared by using *t* tests. There were no significant differences in mean daily gluten intakes between the children who developed CD and those who did not develop CD ($P > 0.05$). Additional information is shown in Supplemental Table 2. CD, celiac disease.

show an association with the risk of CD. Only the *HLA* risk groups 3 and 5 showed a significantly lower risk of CD development compared with *HLA* risk group 1, which was the group with the highest risk (12). HRs and *P* values obtained by the univariable and unadjusted Cox proportional hazards regression model were similar to those obtained with the multivariable and adjusted model, except for the variable estimated MDGI at 18 mo of age (HR: 1.06; 95% CI: 1.01, 1.12; $P = 0.02$; data not shown).

Moreover, this model included the interaction between *HLA* risk group, gluten consumption pattern, and CD development risk. This analysis showed that the risk of CD development in *HLA* risk groups 1, 3, 4, and 5 had no significant interaction with gluten consumption pattern (*P* values of HRs = 0.99, 0.11, 0.2, and 0.88, respectively). Only *HLA* risk group 2 (*HLA-DQ2.2/-DQ7*) showed a significant interaction with gluten consumption pattern and the risk of CD development ($P = 0.031$). The risk increased with the sharp monthly increase in gluten consumption at age 11–18 mo (i.e., with a steeper initial slope) (Table 3, **Supplemental Figure 3**).

TABLE 3
Effect of the variables and interactions included in the multivariable regression model on the development of celiac disease[1]

Variable	HR (95% CI)	*P*
Country		
Spain	0.62 (0.21, 1.79)	0.37
Germany	1.0 (Reference)	—
Netherlands	1.03 (0.36, 2.94)	0.96
Hungary	1.03 (0.35, 2.99)	0.96
Italy	0.42 (0.11, 1.61)	0.21
Sex		
Female	1.0 (Reference)	—
Male	0.66 (0.38, 1.16)	0.22
Intervention group		
Gluten	1.0 (Reference)	—
Placebo	0.86 (0.49, 1.50)	0.59
HLA risk group		
Group 1	1.0 (Reference)	—
Group 2	0.35 (0.11, 1.01)	0.07
Group 3	0.19 (0.08, 0.46)	<0.001*
Group 4	0.35 (0.11, 1.10)	0.40
Group 5	0.18 (0.05, 0.68)	0.01*
Gluten consumption pattern		
Initial slope	1.0 (Reference)	—
Estimated MDGI at 18 mo	0.98 (0.89, 1.09)	0.76
Final slope	1.17 (0.59, 2.31)	0.66
Interaction of variables		
Initial slope		
HLA group 1	1.0 (Reference)	—
HLA group 2	5.81 (1.18, 28.74)	0.031*
HLA group 3	2.31 (0.63, 8.4)	0.20
HLA group 4	0.04 (0.01, 2.19)	0.11
HLA group 5	1.18 (0.14, 9.63)	0.88

[1] Numbers (*n*) for each variable are shown in Table 1. The Cox proportional hazards regression model was adjusted to assess the association of the variables of country, sex, intervention group, gluten consumption pattern (including values of MDGI), *HLA* risk group, and the interaction between *HLA* risk group and gluten consumption pattern with the risk of celiac disease development. *P* values for the mixed models were estimated by using Satterthwaite's approximation to the df. *Significant, $P < 0.05$. *HLA*, human leukocyte antigen; MDGI, mean daily gluten intake.

DISCUSSION

The main finding of our study is that the amount of gluten consumed between 11 and 36 mo of age is not related to the development of CD by age 6 y in a cohort of children with an increased genetic risk of the disease. Despite the differences in MDGI among countries, the amount of gluten consumed at all ages and in each country was similar between the children who developed CD and those who did not. Moreover, if the consumption of high amounts of gluten increased the risk of CD development, we would expect Spain to be the country with the lowest percentage of CD cases and Italy and Hungary with the highest number of CD diagnoses, which was not the case.

Our findings differ from those of previous studies based on an epidemic of CD in Sweden, which suggested that the ingestion of larger amounts of gluten at the time of gluten introduction increased the incidence of CD before the age of 2 y (4). An important contributing factor to the increase in CD cases during the years of the epidemic (1987–1995) was that daily gluten consumption in children <2 y of age during the epidemic was double (47 g flour/d) that of the pre-epidemic period (25 g flour/d) (4). However, the authors did not specify intakes for different age ranges (4). According to the provided information, which was estimated from the amount (tons) of gluten-containing formula sold per year, this means an average consumption of ~2.5 g (pre-epidemic) and 4.5 g (epidemic) gluten/d. In contrast, in our cohort, gluten intake at an early age had no impact on CD development before the age of 2 y, even with higher MDGIs than in the Swedish epidemic. For example, the percentage of CD cases <2 y in Hungary, the country with the highest gluten intake at 1 and 2 y of age, was lower than in Spain, the country with the lowest gluten intake at 1 and 2 y of age (Table 2).

Our results also differ from the recent findings in a nested case-control study performed in The Environmental Determinants of Diabetes in the Young (TEDDY) Swedish cohort (16, 23). With the use of a 3-d FR, the authors reported that the Swedish CD cases consumed a greater amount of gluten at the visit before seroconversion to positive TG2A than did their controls who were matched for year of birth, sex, and *HLA* risk group (4.9 and 3.9 g/d, respectively) (16). The authors concluded that the quantity of gluten intake before 2 y of age modulates the risk of CD in Swedish children. However, contrary to our study, in the TEDDY study as well as in a previous study that addressed the amount of gluten ingestion at the time of weaning (5), gluten intake was categorized by tertiles of quantity and, the categorization of continuous variables has well-known shortcomings (24, 25). The relation between the response (CD development) and the predictor (amount of gluten ingestion) is horizontal within intervals; in the extreme ranges of the variable, the outer intervals will be broader, resulting in significant heterogeneity of subjects within those intervals and an increased risk of residual confounding. In addition, the optimal cutoffs are not reproducible across studies. Because our data were not categorized, we believe that our results are more reliable than those in previous reports.

An intriguing finding in our study was the relation between the sudden monthly increase in gluten intake from 11 to 18 mo of age and a significantly increased risk of CD in *HLA-DQ2.2/-DQ7*–positive children (*HLA* risk group 2) but not in the other *HLA* risk groups. Thus, a lower monthly increase in gluten intake

between 11 and 18 mo of age seems to be a modulating factor for this specific *HLA* genotype. Recent publications have shown that *HLA* risk group 1 (DQ2.5/DQ2.5 and DQ2.5/DQ2.2) has a higher intrinsic risk of CD development at early ages than do the other *HLA* groups (12, 13), and a CD risk gradient related to *HLA* genotype has been established. It is possible that, for the highest-risk genotype (*HLA* group 1), environmental factors, such as gluten intake, might have only a tangential role, whereas for individuals with lower genetic risk (i.e., DQ2.2/DQ7), additional, nongenetic factors, such as gluten ingestion, may have a more relevant modulatory effect. The potential role of a higher antigenic load or a larger immunogenic repertoire of peptides in this genotype (DQ2.2/DQ7) is also a possibility (26). Whatever the reason, our study shows that there is a relation between certain *HLA* genotypes, gluten consumption, and the risk of developing CD and supports that other genetic and environmental factors are also involved in the development of CD. Another interesting finding in our study is that boys showed a trend toward a higher gluten consumption than girls at all ages, even if in this cohort as well as in other prospective cohorts of young children with CD, girls have a higher risk of CD development at a very young age (12, 27–29).

A strength of our study is the inclusion of a large number of children from different European countries, together with a prospective study design and the statistical analysis performed. We were thus able to prospectively obtain precise data on gluten consumption at the potential relevant ages, namely from 4 to 36 mo of age, which provides detailed information not reported in previous studies that included retrospective estimations or categorized data. In addition, for the first time to our knowledge, this enabled us to establish gluten consumption patterns in children at risk of CD from different European countries.

One possible limitation is the gradual introduction of gluten at ≤10 mo of age, which is not representative of gluten introduction in real life. However, the goal of the PreventCD study was to induce tolerance by feeding small amounts of gluten from 4 to 6 mo. Because data from observational trials indicated that high amounts of gluten after weaning might be harmful, we believed for ethical reasons to restrict the gluten intake of all participants to small amounts from ages 7 to 10 mo, with unrestricted intakes thereafter. Although all of the parents received written instructions on the stepwise increase in gluten intake, we cannot exclude that some parents did not adhere strictly to the recommendations. However, the statistical method applied takes into account this potential error in the results. Another possible shortcoming is that we only assessed the incidence of CD until the age of 6 y. However, this period shows the highest incidence and it is less likely that dietary habits in early infancy could influence CD that starts late in childhood.

In conclusion, our data suggest that the amount of gluten as well as gluten consumption patterns in early life have no impact on CD development at ≤6 y of age. Our findings show that only in children with *HLA-DQ2.2/-DQ7* genotypes do high intakes of gluten increase the risk of CD, and this group should be confirmed as an independent cohort. Our results support the role of other, so far unknown, environmental factors in CD development.

The authors' responsibilities were as follows—PC-E: had full access to all of the data in the study and had responsibility for the integrity of the data and the accuracy of the data analysis; PC-E, MLM, and CR-K: designed the research and had primary responsibility for final content; PC-E, MLM, RA, GC, JG, EM-O, KW, SLV, IRK-S, IP, RT, S Koletzko, and CR-K: conducted the research; DH and ES: analyzed data; and all authors: wrote the manuscript and provided critical revision and important intellectual content, and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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