Mode of Delivery and Risk of Celiac Disease: Risk of Celiac Disease and Age at Gluten Introduction Cohort Study

Elena Lionetti, MD, PhD[1], Stefania Castellaneta, MD[2], Ruggiero Francavilla, MD, PhD[3], Alfredo Pulvirenti, PhD[4], and Carlo Catassi, MD, MPH[1,5], for the SIGENP Working Group of Weaning and CD Risk*

Objective To determine whether the mode of delivery is associated with the risk of celiac disease (CD) in a cohort of children genetically predisposed to CD prospectively followed from birth.

Study design By telephone interview, we recorded information on the mode of delivery of children participating in the Risk of Celiac Disease and Age at Gluten Introduction study, a multicenter, prospective intervention trial that compared early and delayed introduction of gluten in infants with at least 1 first-degree relative affected with CD. The human leukocyte antigen genotype was determined at 15 months of age, and serologic screening for CD was performed at 15, 24, and 36 months of age and at 5, 8, and 10 years of age. Patients with positive serologic findings underwent intestinal biopsy. The primary outcome of the current study was the prevalence of CD autoimmunity and overt CD at 5 years of age, according to the mode of delivery.

Results The study-group included 553 children at CD risk because of positivity for human leukocyte antigen-DQ2, -DQ8, or both. We obtained data on the mode of delivery from 431 of 553 children; 233 of 431 children were born by vaginal delivery (54%). At 5 years of age, the prevalence of CD autoimmunity or overt CD was not different between children born by cesarean or vaginal delivery (24% and 19%, $P = .2$; 19% and 14%, $P = .2$ respectively, by the log-rank test).

Conclusions In this cohort of children genetically predisposed to CD, the mode of delivery did not influence the risk of developing CD. *(J Pediatr 2017;184:81-6).*

Celiac disease (CD) is a systemic immune-mediated disorder caused by the ingestion of gluten-containing grains in genetically susceptible persons.[1] Human leukocyte antigen (HLA)-DQ2 and/or -DQ8 genotypes play a major role in CD predisposition.[2] However, the complex interplay between genetic and environmental factors regulating the balance between tolerance and immune response to gluten is still poorly understood. The prevalence of CD has increased in developed countries over recent decades, supporting the role of 1 or more environmental triggers other than gluten.[3] It has been recently hypothesized that intestinal infections, the amount and quality of ingested gluten, the composition of intestinal microbiota, the mode of delivery, and infant-feeding practices are all possible triggers of the switch from tolerance to an abnormal immune response to gluten.[1]

The Risk of Celiac Disease and Age at Gluten Introduction (CELIPREV) study is a multicenter, prospective study investigating the interplay between environmental and genetic factors on the development of CD in a cohort of infants with a family risk of CD, followed on from birth to at least 5 years of age.[4,5] By analyzing the rate of CD autoimmunity and disease development in this study group, we recently showed that several factors related to early infant nutrition, particularly breastfeeding and early (6 months) vs delayed (12 months) gluten introduction, do not modify the risk of CD. In the same study, we also showed that predisposing HLA gene dosing is the most influential variable in increasing the risk to develop CD.[4] At the time the study was designed, the knowledge on possible environmental "modulators" of CD risk was mostly restricted to the pattern of infant nutrition and infections. For this reason, the investigation of other factors, such as type of delivery, was not included in the original study design.

CD	Celiac disease
CELIPREV	Risk of Celiac Disease and Age at Gluten Introduction
HLA	Human leukocyte antigen
HR	Hazard ratio
MoBa	Norwegian Mother and Child
TGA2	IgA antitransglutaminase type 2

From the [1]Departments of Pediatrics, Marche Polytechnic University, Ancona, Italy; [2]Department of Pediatrics, San Paolo Hospital; [3]Department of Developmental Biomedicine, University of Bari, Bari, Italy; [4]Department of Clinical and Molecular Biomedicine, University of Catania, Catania, Italy; and [5]Center for Celiac Research, MassGeneral Hospital for Children and the Celiac Program, Harvard Medical School, Boston, MA

*List of additional members of the SIGENP Working Group of Weaning and CD Risk is available at www.jpeds.com (**Appendix**).

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Preliminary results of this study were presented as an oral communication during the Annual Meeting of the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition, Athens, Greece, May 25-28, 2016, and has been accepted for oral presentation to the World Congress of Paediatric Gastroenterology, Hepatology and Nutrition, Montreal, Canada, October 5-8, 2016.

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In recent years, there have been controversial reports on the influence of delivery mode on the risk of CD development. In 2010, Decker et al[6] first reported an increased risk of CD in infants delivered by cesarean delivery. In contrast, Roberts et al[7] found a decreased risk of CD after cesarean birth. In a population-based nationwide study, Marild et al[8] showed a positive association of CD with elective, but not emergency, cesarean delivery. Finally, the Norwegian Mother and Child (MoBa) cohort study found no association between CD and the mode of delivery.[9] All these studies were flawed by a detection bias, because only patients with clinically detected CD were taken into consideration.

In the present study, we aimed to determine whether the mode of delivery was associated with the risk for CD in genetically predisposed children who participated in the CELIPREV study. Because all children were periodically screened for CD serologic autoimmunity markers, our study included both symptomatic and subclinical disease cases.

Methods

The CELIPREV is a prospective, multicenter, nationwide intervention trial that was primarily aimed to evaluate the role of age at gluten introduction (6 vs 12 months) on CD development, in a large cohort of children at family risk of CD, that were followed from birth.[4] Family risk was defined by having at least 1 first-degree relative affected with CD. Newborns were recruited in 20 centers scattered throughout Italy between October 2003 and January 2009. Infants were assigned to introduce gluten-containing food at either 6 (group A) or 12 months of age (group B) by block randomization and followed up for at least 5 years.

The first work-up for CD was performed at 15 months of age (IgA antitransglutaminase type 2 [TGA2], IgA antigliadin antibodies, total IgA, and HLA-DQ2 and HLA-DQ8 genotype), then at 24 months (TGA2 and antigliadin antibodies), and at 3, 5, 8, and 10 years of age (TGA2). Children with IgA deficiency (IgA <5 mg per deciliter), were screened by determination of IgG antigliadin antibodies. Subjects with a positive CD serology determination were recalled for test repetition and evaluation of serum IgA class endomysial antibodies. A small intestinal biopsy was recommended to all children with 1 of the following results: (1) positive results for TGA2 and endomysial antibodies; (2) positive results for IgG antigliadin antibodies in association with IgA deficiency; or (3) positivity of IgA antigliadin antibodies in children aged less than 2 years.

Characteristics of the CELIPREV cohort have been described previously.[4] After exclusion of 125 patients who dropped out, the cohort included 707 infants. Of them, 154 were negative for of HLA-DQ2 and HLA-DQ8 and were excluded from further follow-up. The final study group included 553 children who were positive for HLA-DQ2, HLA-DQ8, or both.[4]

For the purpose of the present study, we contacted all the families of the 553 children with a predisposing genotype by a telephone interview to record information on the mode of delivery, categorized as either cesarean or vaginal delivery. The institutional review board of the coordinating center (Università Politecnica delle Marche, Ancona, Italy) approved this study protocol, registered on ClinicalTrials.gov CELIPREV, number, NCT00639444. Written informed consent was obtained from the parents or guardians of the children.

HLA Genotyping

The detection of HLA alleles was performed by the DQ-CD Typing Plus kit (BioDiagene, Palermo, Italy). On the basis of HLA determination, children were classified as having no risk of CD (the absence of HLA-DQ2 and HLA- DQ8), a low risk of CD (HLA DQ 2.2: a single or double copy of the DQB1*02 allele associated with DQA1 alleles different from the DQA1*05), a moderate risk of CD (HLA DQ8: DQA1*03-DQB1*0302/0305), a high risk of CD (a single HLA DQ2.5: DQA1*05-DQB1*02 in either cis or trans position), a highest risk of CD (homozygosity for HLA DQ 2.5: double copy of DQA1*05-DQB1*02.[10,11]

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 with the use of a commercial kit (Menarini Diagnostics, Florence, Italy). More than 20 arbitrary units indicated a positive result. IgA and IgG antigliadin antibodies were measured by means of enzyme-linked immunosorbent assay 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.

Small-Bowel Biopsies

Small-bowel biopsies were performed by means of upper endoscopy, and at least 4 specimens were obtained from the bulb and the descending part of the duodenum. Lesions in the small intestine were graded at the coordinating center in Ancona, Italy, according to the Marsh classification.[12] We defined overt CD as CD autoimmunity and a Marsh classification of 2 or 3 at small-bowel biopsy (on a scale of 0-3, with higher scores indicating villous atrophy), and potential CD as CD autoimmunity and a Marsh classification of 0 or 1.[4]

Outcome Measures

The primary outcome of the current study was the prevalence of CD autoimmunity and overt CD at 5 years of age according to the mode of delivery. Secondary outcomes were (1) the prevalence of overt or potential CD according to the mode of delivery; and (2) the interplay between the mode of delivery and the available nutritional and genetic factors (breastfeeding, age at gluten introduction, genotype, sex, CD-affected first-degree relative, intestinal infections, HLA genotype) in influencing the risk of CD.