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Despite some recent reports showing no (or a positive) association between breastfeeding and later celiac disease (CD), most data suggest that breastfeeding at the time of gluten introduction reduces the risk to develop CD

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Timing of Introduction of Gluten and Celiac Disease Risk

by Jonas F. Ludvigsson and Alessio Fasano

Key insights

Infant nutrition plays an important role in celiac disease (CD) development. Although most studies suggest breastfeeding reduces the CD risk, further research is needed to clarify the role and timing of gluten introduction, e.g. for infants with first-degree relatives with CD.

Current knowledge

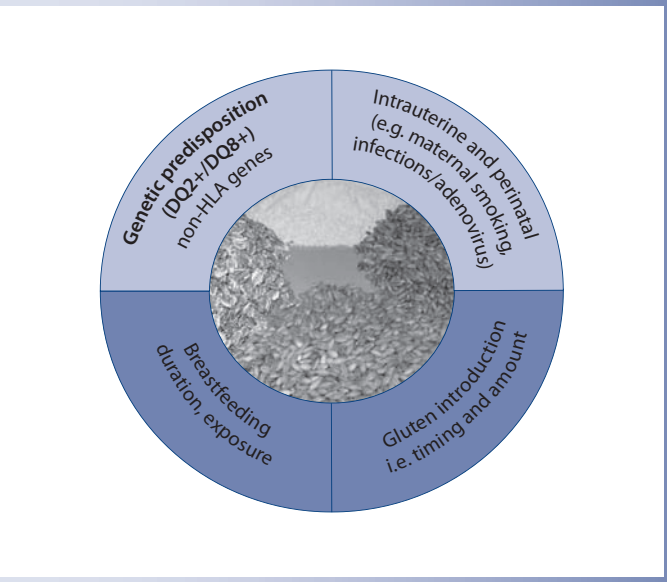
CD is an autoimmune disease, originating in the upper part of the small intestine, characterized by an abnormal immune response towards dietary gluten in genetically susceptible individuals (e.g. haplotypes DQ2 or DQ8; further genetic components are implicated). Additional non-dietary risk factors include intrauterine and perinatal factors. Current data suggest that breastfeeding at the time of gluten introduction reduces the risk of future CD. However, it is still unclear if any protective effect of breastfeeding is persistent or simply delays CD onset.

Practical implications

The considerable interplay between the potential risk factors and the events during an infant's early life make CD prevention a challenge. Careful study design and analysis are needed to understand the relative risks, based on the outcomes of ongoing and planned studies.

Recommended reading

Ivarsson A, Hernell O, Stenlund H, et al: Breast-feeding protects against celiac disease. Am J Clin Nutr 2002;75:914–921.



Identified (bold) and potential risk factors for the development of CD. HLA = Human leukocyte antigen (see text for details).

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Timing of Introduction of Gluten and Celiac Disease Risk

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Key Messages

- Infant nutrition affects the risk of developing celiac disease.
- Most studies suggest that breastfeeding reduces the risk of future celiac disease.
- Data on the impact of complementary feeding on celiac disease are inconsistent.

Key Words

Autoimmunity · Breastfeeding · Celiac disease · Gluten

Abstract

Breast milk is the natural nutrition for infants, but in the second half of the first year of life, complementary feeding is needed. Many complementary foods contain gluten, but gluten exposure is associated with the risk of developing celiac disease (CD). CD is a disease with considerable morbidity and mortality. Although CD is associated with certain genetic features, carrying the human leukocyte antigen haplotypes DQ2 or DQ8 (a prerequisite for CD development) cannot fully explain who will or who will not develop CD. Potential risk factors for CD include perinatal events and infant feeding practice. With the exception that children who are breastfed at and beyond gluten introduction into the diet probably may be at a lower risk of developing CD, and that heavy gluten load early in life may increase the risk of future CD, data on the impact of infant feeding are inconsistent.

Introduction

Celiac disease (CD) is a lifelong immune-mediated disease that occurs in 1–2% of Western populations [1–3]. CD originates in the upper part of the small intestine and is characterized by villous atrophy and inflammation of the duodenum and/or jejunum [4–6] (fig. 1). The disease develops as a consequence of gluten exposure in some genetically predisposed individuals carrying the human leukocyte antigen (HLA) DQ2/DQ8 haplotypes [7], and is associated with considerable mortality [8] and morbidity [9, 10]. In recent years, it has become evident that CD is not only a disease of the Western world [1] but also occurs with similarly high prevalence in some developing countries [11–16].

In this paper, we review several factors that impact on the risk to develop CD, with special emphasis on the role of breastfeeding and timing of gluten introduction in CD development. The importance of these factors is demonstrated by the epidemic of CD experienced in Sweden in the mid-1980s, in part triggered by the mode (timing and dose) of gluten introduction in infants' diet (fig. 2) [17, 18].

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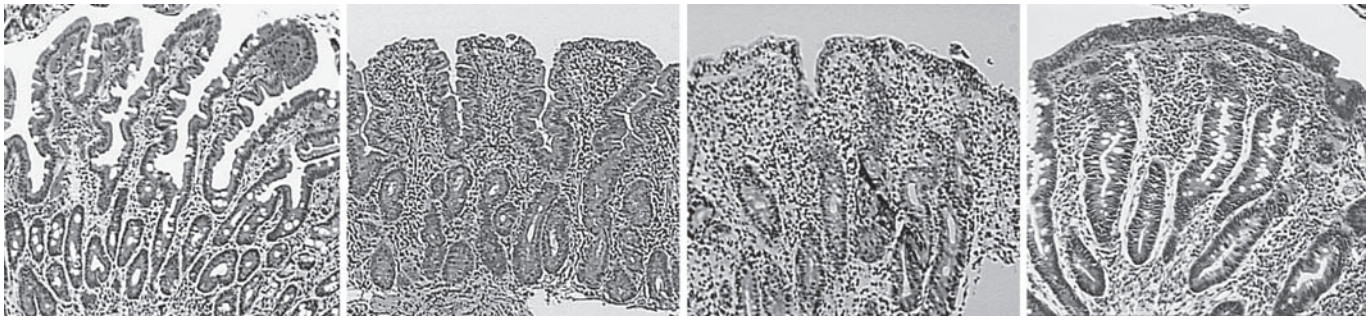

Fig. 1. Histopathology of the small intestine.

Genetics

CD is a multifactorial condition with unparalleled evidence of the pivotal role of HLA DQA1*05-DQB1*02 (DQ2) and DQA1*03-DQB1*0302 (DQ8) in disease predisposition [19]. The most likely mechanisms to explain the association of CD pathogenesis with HLA class II genes is that these DQ molecules bind gliadin peptides to present them to T cells. Mapping of this binding has clarified the importance of proline spacing and gliadin peptide deamidation in this process [20]. However, HLA alone does not explain genetic susceptibility because the concordance rate between identical twins (approximately 70%) is higher than that between HLA identical siblings (30%) [21], suggesting that additional genetic factors influence disease propensity. Recent genetic studies have identified 39 non-HLA risk genes, mostly related to immune responses (both innate and adaptive immune responses), inflammation, and intestinal barrier function [22]. Several genes present in the extended HLA complex have been implicated in CD predisposition. Associations with the tumor necrosis factor (TNF)-2 allele have also been reported, the polymorphism of which is associated with increased TNF-α expression [23]. A series of whole genome screening studies has been performed in patients with CD. The data regarding specific genes or genome areas possibly involved have not been consistently replicated in different population groups, suggesting an inter-population variation of extra-HLA genes associated to CD. The region that has most consistently been linked to CD is located on the long arm of chromosome 5 (5q31–33) [24]. There is also evidence for susceptibility factors on chromosome 19 [25]. Fine mapping of the latter region has identified the gene *MYO9b*, coding for a myosin probably involved in actin-based processes and, therefore, intestinal barrier regulation [26]. Taken together, it is possible that non-*HLA* genes together contribute more to the genetic susceptibility for CD than the identified HLA genotypes do, but the contribution from each single, predisposing non-*HLA* gene appears to be modest.

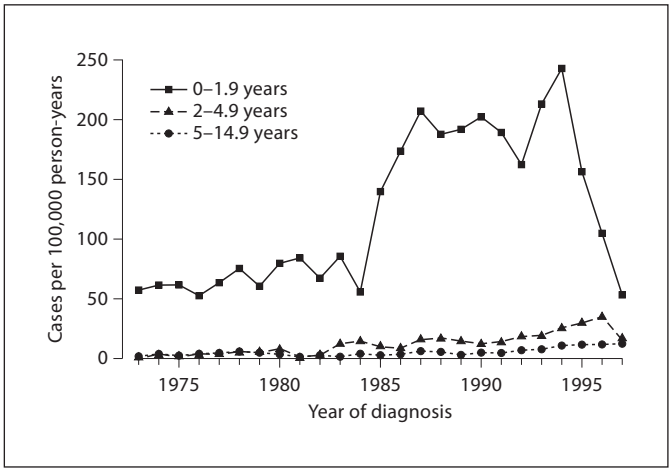

Fig. 2. The 'Swedish epidemic' of CD. Differences in the incidences of CD in Sweden over time [reprinted with permission from ref. 17].

The data regarding specific genes or genome areas possibly involved have not been consistently replicated in different population groups, suggesting an inter-population variation of extra-HLA genes associated to CD.

Non-Dietary Risk Factors for CD

Several reports have shown that intrauterine and perinatal conditions influence the risk of CD. In 2002, Sandberg-Bennich et al. [27] reported that both neonatal infections [odds ratio (OR) = 1.52; 95% confidence interval (CI) = 1.19–1.95) and being small for gestational age (OR = 1.45; 95% CI = 1.20–1.75) increased the risk of CD.

Although several studies have shown smoking to be associated with a lower risk of CD, maternal smoking may actually increase the risk of CD in the offspring [26]. The detrimental effects of maternal smoking with regard to CD risk has since been confirmed in two more recent studies [28, 29], reaching statistical significance in the largest study [27] but not in the second one [28].

To date, it is unclear whether a cesarean section increases [30] or decreases [28] the risk for CD in the offspring. The hypothesis behind the cesarean section leading to a higher risk of CD is that such infants are not exposed to the maternal vaginal flora (that may establish a 'tolerogenic' gut microbiome), since they do not pass through the birth canal.

Several reports have shown that intrauterine and perinatal conditions influence the risk of CD.

Since Kagnoff et al. [31] reported in their landmark paper that adenoviruses may be involved in the pathogenesis of CD, researchers have tried to identify the role of infectious disease in its pathogenesis. The parallel findings of a potential role of virus infections in type 1 diabetes, another autoimmune disease that also confers increased risk for CD [32–34], has raised this interest. At least 4 studies have evaluated birth season and CD [35–38], with the 2 largest studies [36, 37] suggesting that being born during spring-summer months may be associated with an increased CD risk in children. Evidence is less clear when actual infections are examined. A German study found that children with CD had more often experienced earlier gastrointestinal (GI) disease (including both infections and non-infectious disease) prior to CD diagnosis (adjusted OR = 2.97; 95% CI = 1.08–8.14) [30]. However, this study was based on retrospective data; recall bias mixed with actual CD symptoms preceding CD diagnosis cannot be ruled out as an explanation [30]. In contrast, Welander et al. [39] used prospectively collected data to examine the role of infectious disease prior to CD diagnosis in children. Although 40.9% of children with CD had an infection at the time of gluten introduction compared with 26.8% of children without a CD diagnosis ($p = 0.035$), the difference diminished after adjustment for age at gluten introduction and breastfeeding duration (adjusted hazard ratio = 1.8; 95% CI = 0.9–3.6; $p = 0.111$) [39]. For gastroenteritis at the time of gluten introduction, the adjusted hazard ratio was higher (2.6) but the 95% CIs were wide due to insufficient power (0.2–30.8) [39]. A US study found that children with at least 2 rotavirus infections had an increased risk of CD but only when the data were analyzed using tests for trend ($p = 0.037$) [38]. The unadjusted rate ratio for developing CD after at least 2 rotavirus infections was 3.76 (95% CI = 0.76–18.7; with $p = 0.244$ when re-calculated by us using Fisher's exact test) [38].

Breastfeeding

Several studies on breastfeeding and CD suggest that breastfeeding reduces the risk for future CD [40]. However, randomized trials are lacking and with few exceptions [28, 39, 41], existing studies have been based on retrospectively collected data [42–47]. The largest case-control study so far is that by Ivarsson et al. [46], where the authors found a reduced risk of future CD in children aged 0–1.9 years at diagnosis but not in children aged 2.0–14.9 years at diagnosis. The protective effect in children <2 years was stronger in those being breastfed beyond the introduction of gluten (OR = 0.36; 95% CI = 0.26–0.51). Two other studies [44, 46] found similar results while a case-control study controlling for HLA status found no association between breastfeeding at the time of gluten introduction and CD; however, this study was only based on 8 cases with silent CD [45].

In contrast, the most recent case-control study by Decker et al. [30] reported opposite results. In their study, breastfeeding was associated with a statistically significant increase for CD (OR = 1.99, 95% CI = 1.12–3.51) [29]. However, it should be underlined that this was a secondary analysis, as the primary objective of the Dekker et al. study [29] was to examine the risk of pediatric gastrointestinal disease in offspring to mothers undergoing cesarean section.

The US study by Norris et al. [41] followed up 1,560 children with HLA-DR3 or -DR4 through regular blood tests and questionnaires. The main outcome was CD autoimmunity, which required either 2 positive CD serology blood tests or 1 positive blood test and 1 small intestinal biopsy consistent with CD. Some 49% (25/51) of chil-

But the exposure in the British study was 'ever breastfed' and therefore added little information to whether breastfeeding at the time of gluten introduction protects against CD or not.

dren with CD autoimmunity were breastfed when first introduced to wheat, barley or rye compared with 44% (660/1,509) in controls ($p > 0.05$). The actual breastfeeding duration was longer in children with CD autoimmunity (median duration: 8.8 months) than in controls (6.8 months) [41]. One of the main strengths of this paper is that the authors screened their population for CD [40]. Many other studies have only looked at symptomatic CD leading to a physician diagnosis [18, 28, 39] and it could then be argued that their findings apply only to CD symptoms, or that breastfeeding may postpone the onset of symptoms [18] and lead to fewer diagnosed cases with CD early in life.

In a Swedish study, Welander et al. [39] used prospectively collected data from the ABIS (All Babies in Southeast Sweden) study and detected no association between breastfeeding duration and subsequent development of CD. The authors also found no association between breastfeeding duration and later CD when taking age at gluten introduction and prospectively recorded infections at the time of gluten introduction into account. Actually, the authors reported a trend towards early ending of breastfeeding being protective against CD (e.g. risk of later CD in offspring to mothers ending breastfeeding at 0–2 months of infant age: OR = 0.7; at 3–4 months: 0.7, and at 4–5 months: 0.3). However, it should be noted that none of these risk estimates reached statistical significance [39].

In a British study by Roberts et al. [28], the authors used data from the Oxford record linkage study. That study found no difference in the cumulative incidence of CD among breastfed (32.4/100,000) and non-breastfed infants (43.2/100,000; $p = 0.28$). But the exposure in the British study was 'ever breastfed' and therefore added little information to whether breastfeeding at the time of gluten introduction protects against CD or not.

To summarize, most studies point toward a protective effect of breastfeeding against CD, but it is unclear if this protective effect is persistent or only represents a delay in the diagnosis of CD [48]. One of the mechanisms potentially explaining an inverse relationship between breastfeeding and CD is that continuing breastfeeding could reduce the amount of gluten given [18]. Human milk IgA antibodies could also decrease the immune response against gluten.

Timing of Gluten Introduction

Two large Swedish studies have both failed to show any association between timing of gluten introduction and risk of CD [39, 18]. Introducing gluten at the age of 5–6 months did not increase the risk of CD in the study by Ivarsson et al. [18] (OR = 1.4; 95% CI = 0.87–2.4). Furthermore, this disappeared in the multivariate analysis as an independent risk factor. Using the age of 5–6 months as a reference when estimating hazard ratios for future CD, Welander et al. [39] showed that there was no difference in the risk of CD in infants aged 3–4 months or 7–8 months at gluten introduction. Other studies confirm the lack of an association between timing of gluten introduction and later CD [42–44, 46, 48].

The one study that showed an association between age at gluten introduction and CD is that by Norris et al. [40]. Using the age of 4–6 months as the reference category for gluten introduction, infants who first consumed gluten at the age of 1–3 months were at a 23-fold increased risk of CD; those who consumed gluten after the age of 6 months were at a 4-fold increased risk of CD [41]. It should, however, be emphasized that the number of children developing CD in those exposed to gluten before 4 months of age was small (3 children developed CD in this category, as opposed to an expected '0.13 child') [41].

It should, however, be emphasized that the number of children developing CD in those exposed to gluten before 4 months of age was small.

Amount of Gluten

The mean intake of gluten in a pediatric cohort in the Netherlands was around 1 g at 6 months of age, rising fast to 6 g/day at the age of 8 months and then remaining at that level until 12 months of age [49]. It seems likely that the amount of gluten influences the risk of CD [18]. In a Swedish study, children with CD had significantly more often been given large (as opposed to

'small-medium') daily amounts of gluten 2 weeks after gluten introduction compared to age- and sex-matched controls. But this association was only seen in children diagnosed before 2 years of age [18]. There was no difference in the amount of flour introduced in the first portion between cases with CD and healthy controls [18]. Several authors have also implicated divergent flour consumption as an explanation for the geographic differences in CD incidence in Europe [50–52]. Furthermore, it is known that the amount of gluten in the diet correlates with the mucosal damage [53, 54], and we speculate that large amounts of gluten in the early non-breast milk diet could trigger more symptoms in children with CD and, thus, a diagnosis of CD. We have also shown that adults with CD may tolerate up to 50 mg/day of gluten without mucosal injury [55].

Furthermore, it is known that the amount of gluten in the diet correlates with the mucosal damage [52, 53], and we speculate that large amounts of gluten in the early non-breast milk diet could trigger more symptoms in children with CD and, thus, a diagnosis of CD.

Ongoing Studies

As previously summarized, several retrospective studies have suggested that the age at gluten introduction in the diet of infants at risk for CD may affect the incidence of the disease. However, the data supporting this hypothesis are circumstantial, limited by their retrospective design, and often criticized by alternative interpretations suggesting that the delay in gluten exposure merely postpones the onset of symptoms rather than prevents the disease. Due to the cross-sectional design of these studies, it remains unclear whether the reported microbial associations (see below) are pathogenic or merely the consequence of CD intestinal inflammation. In order to clarify the role of infant nutrition on the risk of CD development, at least two prospective, intervention studies have recently been initiated. The results of these long-term studies will be available within the next few years.

The PreventCD Family Study

This prospective, double-blind, placebo-controlled study is currently ongoing in 10 European countries and a total of 1,000 children are involved [56]. The participating children and mothers are to be followed for a period of 1–3 years. The project will study the influence of early diet on CD prevention. The general concept is that small amounts of food substances are administered gradually to 'teach' the immune system not to respond to this foodstuff. This is also called 'desensitization' or 'induction of oral tolerance'. After HLA-typing, DQ2 and/or DQ8+ newborns from families at risk of CD are randomized and blindly allocated to intervention with either 100 mg gluten or placebo (lactose). After 6 months of age, gluten is gradually introduced into their diet. CD autoantibodies are then monitored every 3–6 months to disclose gluten sensitization.

The Italian Baby Study

This is another initiative aimed at evaluating the role of (a) age at gluten introduction on CD-related autoimmune serological changes in a large cohort of at-risk infants (first-degree relatives of patients with CD); (b) other early environmental factors, particularly the role of breastfeeding; (c) different HLA-DQ2/DQ8 genotypes (high risk vs. low risk) on CD predisposition, and their interplay with infant nutrition patterns. Between October 2004 and June 2007, 729 infants (51% male) at increased risk for CD were enrolled in this prospective, multicenter intervention study conducted in Italy. At weaning, infants were assigned to gluten introduction into their diet either between the 4th and 6th month (group A) or after the 12th month (group B), then entered a follow-up period of 5 years. Diet (duration of breastfeeding, type of formula, adherence to the dietary plan and amount of gluten ingested) and clinical data were collected during telephone or face-to-face interviews at 4, 7, 9 and 12 months of age. CD serology (IgA anti-transglutaminase antibodies) was performed at 15 (plus HLA-DQ genotype), 24, 36 and 60 months of age. Small intestinal biopsy was recommended for all infants showing positivity of CD serological tests. At the last study update (September 2011), 100% of children had completed the 36-month follow-up. Fifty-two percent of infants were enrolled in group A and 48% in group B. Prevalence of biopsy-proven CD at 24 months was 8% in group A and 2% in group B ($p < 0.01$). However, this difference decreased at the 36-month follow-up, when the prevalence of CD became similar in the two groups (approximately 10%). Combined, these results suggest that delaying gluten introduction merely delays the onset of CD rather

than prevents it. However, there was no increased risk of developing CD as previously reported [40].

Intestinal Microbiota and Onset of CD

One follow-up study of the intestinal colonization processes of gut microbiota was conducted in 20 Swedish children stratified by high, intermediate and low genetic risk of developing CD. The total bacterial proportions were significantly higher in the high and intermediate genetic risk group than in the low genetic risk group. Gram-negative bacteria and Bacteroides-Prevotella proportions were higher in the high genetic risk group than in the intermediate and low genetic risk groups. In this study, the analysis of the fecal microbiota was conducted by fluorescence in situ hybridization and flow cytometry [57]. Both phenotypic methods present a substantial amount of variability and may rely on an individual and subjective interpretation, while the 16S rDNA sequencing, based on ribosomal small subunit species-specific variability, has become the qualitative reference technique for bacterial taxonomy and identification [58].

Conversely, our recent prospective studies on the gut microbiome of infants at risk for CD suggest that their microbial ecosystem is different than that of children not predisposed for CD.

In healthy infants, as described by Palmer et al. [58], Bacteroidetes colonize and establish in the GI tract. Although varying from baby to baby in the timing of their first appearance, they are consistently present in nearly all infants by 24 months. The healthy microbiota evolves during different life stages and in infants it shows a lower ratio of Firmicutes to Bacteroidetes than in adults. Overall, the microbial ecosystems in each healthy baby achieve stability converging toward a profile characteristic of the adult GI tract in the first year of life [59]. Conversely, our recent prospective studies on the gut microbiome of infants at risk for CD suggest that their microbial ecosystem is different than that of children not predisposed for CD [Ravel and Fasano, pers. commun.]. Our studies revealed that the colonization process is very dynamic, with a high degree of inter-subject variation over time. Unlike children not predisposed for CD, the GI microbiota of infants at risk for CD does not stabilize towards an adult-like microbiota. Members of the phylum Bacteroidetes are absent from the GI microbiota up to 24 months, while they are predominant in children not predisposed for CD. These data suggest that early dietary and/or probiotic interventions may potentially stabilize the gut microbiota of these at-risk children, so preventing and/or delaying the onset of CD.

Despite some recent reports showing no (or a positive) association between breastfeeding and later CD, most data suggest that breastfeeding at the time of gluten introduction reduces the risk to develop CD.

Conclusion

Breastfeeding is the natural food for infants, but from around 6 months of age, the infant is in need of complementary feeding. The intricate interplay between breastfeeding and gluten introduction (amount and timing) has been the topic of several studies. Despite some recent reports showing no (or a positive) association between breastfeeding and later CD, most data suggest that breastfeeding at the time of gluten introduction reduces the risk to develop CD. It is also plausible that rapidly reaching a comparatively large daily intake of gluten after starting complementary feeding may increase the risk of CD. In the future, when we are able to analyze more carefully the outcomes of some of the ongoing studies, we may come up with refined conclusions, taking also genetic predisposition into account. This may result in evidence-based recommendations on gluten introduction into infants' diet.

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Disclosure Statement

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