

# BMJ Open Infant feeding and risk of developing celiac disease: a systematic review

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## ABSTRACT

**Objective:** To review the evidence for the association of breast feeding, breastfeeding duration or the timing of gluten introduction and the later development of celiac disease (CD).

**Design:** Systematic review.

**Methods:** We searched MEDLINE, via PubMed, EMBASE and Web of Science, for studies published up to 31 August 2015 investigating the association of breastfeeding duration, breast feeding at the moment of gluten introduction or the timing of gluten introduction and the later development of CD. Prospective studies had to enrol infants/children at high risk of CD. For retrospective studies, participants had to be children or adults with CD. The paper quality was assessed by means of a GRADE score and the bias risk was assessed by the Newcastle-Ottawa Scale (for observational cohort studies) and Cochrane Collaboration's tool (for randomised trials).

**Results:** Out of 149 retrieved papers, 48 were considered in depth and 16 were included in this review (9 were prospective and 2 were interventional). We found that neither duration of breastfeeding nor breastfeeding at time of gluten introduction nor the delayed introduction of gluten during weaning were effective in preventing later development of CD.

**Conclusions:** Currently, there is no evidence on the optimal breastfeeding duration or the effects of avoiding early (<4 months of age) or late (≥6 or even at 12 months) gluten introduction in children at risk of CD. Accordingly, no specific general recommendations about gluten introduction or optimal breastfeeding duration can be presently provided on evidence-based criteria in order to prevent CD.

## INTRODUCTION

Celiac disease (CD) is a permanent immune-mediated enteropathy, triggered in genetically predisposed individuals by gluten. Gluten is a protein fraction of cereals, such as wheat, rye and barley. The genetic predisposition consists in the presence of alleles encoding for the molecules DQ2 or DQ8 of the human leucocyte antigen (HLA).<sup>1–3</sup>

CD is probably the best known multifactorial disease. Genetic predisposition and gluten intake are both necessary, but not sufficient for the development of this condition. Only roughly 5% of the DQ2/8+ worldwide

## Strengths and limitations of this study

- Our systematic review offers an inclusive overview of the population-based evidence regarding infant feeding practices and the risk of developing celiac disease (CD).
- A study quality score and a bias risk scale are used to appraise the clinical transferability of each study included in this systematic review.
- A small number of prospective clinical trials are available about the role of breast feeding and the gluten introduction during weaning. The majority of the studies were retrospective and enrolled children not at genetic risk for CD.
- No specific general recommendations about gluten introduction or optimal breastfeeding duration can be presently provided on evidence-based criteria in order to prevent CD. A possible exception might be DQ2 homozygous girls, in whom an early introduction of gluten appears to be associated with a greater risk of subsequent development of CD.

population will eventually develop CD.<sup>4</sup> Therefore, other factors are expected to be involved in CD pathogenesis. Among these, additional genes are increasingly being recognised; but repeated viral infections, modality of delivery, imbalance of the intestinal microbiota and infant feeding practices have also been hypothesised.<sup>5–8</sup>

The hypothesis of inducing, via early feeding practices, oral tolerance to gluten in infants at genetic risk for CD, has been long investigated. Both prolonged breast feeding and gluten introduction during a sensitive 'window' period, in which the infant's immune system is more likely to adapt to food antigens, have been assumed as protective factors towards the development of CD.<sup>8</sup>

The epidemiological evidence about this strategy for the primary prevention of CD is conflicting and definitive recommendations on early feeding in children at genetic risk for CD are not available.

The aim of this paper is, therefore, to systematically review all the related published clinical trials and cohort studies, in order to

assess whether: (1) breastfeeding practice and breastfeeding duration protects from the development of CD; (2) breast feeding at time of gluten introduction exerts a protective effect on CD risk; (3) timing of gluten introduction may have a role in triggering CD; (4) the amount of gluten during the complementary feeding period plays a role in the onset of CD. These evidences are then critically discussed to assess the appropriate timing of first gluten introduction during weaning, also in the light of the role of the other environmental variables.

## MATERIALS AND METHODS

### Search strategy

A systematic review of the literature was initially performed in November 2014, and then repeated in December 2014 and on 1 September 2015. The search was carried out in the content of MEDLINE, via PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) EMBASE and Web of Science, following guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) group. Letters to the editor, abstracts and proceedings from scientific meetings were excluded from the analysis. Only papers in English were considered. Two authors (MS and CA) independently selected the articles, and retrieved and assessed the potentially relevant ones. Discrepancies in article selection were resolved by a face-to-face discussion; if the discrepancy stood, a third researcher was consulted (YS). The following search terms were used: ('celiac' OR 'coeliac' OR 'sprue' OR 'gluten enteropathy') and (breast-feeding OR breastfeeding OR breast feeding OR breastfed); ('celiac' OR 'coeliac' OR 'sprue' OR 'gluten enteropathy') and ('weaning' OR 'complementary feeding'); ('gluten') and ('time OR timing') and ('introduction'); ('gluten') and ('infant feeding') ('celiac' OR 'coeliac' OR 'sprue' OR 'gluten enteropathy') and ('infant feeding').

### Inclusion criteria

#### Types of study

Any type of study on humans, reporting primary data, was included in this systematic review. Previous systematic reviews and meta-analyses were excluded.

#### Types of participants

The prospective studies had to enrol infants/children at increased risk of developing CD. Risk of developing CD was intended as defined by HLA DQ2/8 positivity and/or at least a first-degree relative with CD or type 1 diabetes mellitus (T1DM). For retrospective studies, participants had to be children or adults with CD diagnosed by small bowel biopsy or serological positivity (anti-tissue transglutaminase (tTG) antibodies).

To be included in this analysis, the studies should have assessed the risk of CD in people who were:

- ▶ Ever breast fed compared with those never breast fed;

- ▶ Breast fed for different periods of time;
- ▶ Breast fed at the time of the first gluten introduction during weaning compared with those who were not;
- ▶ Receiving gluten for the first time during weaning at different ages.

### Outcome measures

The primary outcome measures were the development of CD-associated autoimmunity (anti-tTG antibodies) and/or biopsy-proven CD.

### Data collection, extraction and analysis

An initial evaluation of the title, abstract and keywords of every record found was performed by MS and CA. The next step was the retrieval of the full text of potentially relevant trials. The two reviewers independently assessed the eligibility of each potentially relevant trial with the use of inclusion criteria. If they had different opinions, these were resolved by discussion with the third reviewer (YS).

The information extracted included the following: (1) general characteristics of the studies (first author and year of publication of the article, country, number and age of the participants, inclusion and exclusion criteria); (2) design and characteristics of the data collection and eventual intervention; (3) definition of the outcome (CD diagnosis, autoimmunity); and (4) main results. Main summary measure was considered HR/OR.

### Study quality

In order to appraise the quality of the studies included in this review, we used the GRADE score ranging from 0 (minimum) to 4 (maximum) points. GRADE is a systematic and explicit approach to making judgements about quality of evidence, based on a score given to each study according to the following parameters: study design (prospective or observational), allocation process, follow-up, withdrawals, directness consistency and effect size.<sup>9</sup>

### ASSESSMENT OF RISK OF BIAS

For observational cohort studies, we used the Newcastle-Ottawa Scale to assess the risk of bias.<sup>10</sup> This scale uses a star system (with a maximum of nine stars) to evaluate a study in three domains: selection of participants, comparability of study groups and the ascertainment of outcomes of interest. We judged studies that received a score of nine stars to be at low risk of bias, studies that scored seven or eight stars to be at medium risk and those that scored six or less to be at high risk of bias. Similarly, for the randomised trials, we used the Cochrane Collaboration's tool for assessing the risk of bias.<sup>11</sup> This tool evaluates seven possible sources of bias: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. For each individual domain,

we classified studies into low, unclear and high risk of bias.

## RESULTS

### Studies included in the review

Our systematic search through MEDLINE, EMBASE and Web of Science retrieved 149 papers. Of them, 48 papers were potentially considered eligible for inclusion. We obtained the full text of those papers and, after the exclusion of 20 review articles, a commentary and a letter, 11 original research articles were also excluded because they did not meet the inclusion criteria. In detail, the reasons for exclusion were as follows: absence of data about breastfeeding duration and/or the age of gluten introduction, no CD diagnosis or CD autoimmunity as outcome of the study and lack of negative/healthy controls. Thus, 16 papers reporting the results of 16 different studies were included in the analysis.

Of the 16 studies included in this review, nine were prospective (two interventional and seven observational). The interventions consisted of the prescription of gluten introduction (no indication about the amount) at 6 or 12 months of age in the CeliPrev Study<sup>12</sup> and the daily administration of 100 mg of gluten at 16–24 weeks of age in the PreventCD study,<sup>13</sup> respectively.

Three studies were based on populations of children at genetic risk of T1DM.<sup>14–16</sup> Three studies had a multicentric study design.<sup>12 13 16</sup> Most papers (13) were from Europe, with 3 from Italy,<sup>12 17 18</sup> 4 from Sweden,<sup>19–22</sup> 3 from Germany<sup>14 23–24</sup> and 1 each from the UK,<sup>25</sup> Norway<sup>26</sup> and the Netherlands;<sup>27</sup> 1 was multicentric with groups from Europe and the USA,<sup>16</sup> and 1 was a European study funded by the seventh European research framework programme.<sup>13</sup> The remaining study was from the USA.<sup>15</sup>

### Breast feeding and risk of CD

Ten of the 16 papers investigating the effect of breast feeding on the risk of CD concluded that the duration of breast feeding did not show a preventive effect on the development of CD. These studies included the most recent and consequently those with the highest quality score, represented by the two randomised interventional<sup>12 13</sup> as well as eight of the nine prospective studies considered in the analysis.<sup>14–16 22–27</sup> One of these studies even found a positive correlation between prolonged breast feeding for over the first year of age and the increased incidence of CD, although the statistical significance of this association was only minimal.<sup>26</sup> A protective effect of breast feeding on the later development of CD was reported by four retrospective papers (table 1).<sup>17–19 23</sup>

### Breast feeding at gluten introduction and CD

Nine of the 16 studies included in this review examined the effect of breast feeding at the time of gluten introduction on later development of CD (table 2). Among

these, two retrospective studies, enrolling individuals whose genetic background was not studied, as controls, found a preventive effect of being breast fed at the first ingestion of gluten during the introduction of solid foods, with a relevant statistical significance (OR ranging from 0.35 to 0.55).<sup>19 23</sup>

However, none of the six prospective papers did not report such a protective effect.<sup>12 13 15 16 21 26</sup> This group of studies includes the most recent study as well as those with the highest quality score, characteristically including children with a common genetic background (pre-disposing to a risk for CD or T1DM or DQ2/8+ positive children).

### Time of gluten introduction and CD

Eight of the 11 papers reporting information about the time of gluten introduction did not find any correlation between the age of the children at this stage and development of CD (table 3). The recent paper by Lionetti *et al*.<sup>12</sup> concluded that there is no difference in CD incidence at 5 years of age in children who have gluten introduced at 6 months compared with those who have consumed gluten for the first time at 12 months (HR 0.9; 95% CI 0.6 to 1.4). Also, no difference in the incidence of CD between the two groups, by stratifying the children for the genetic risk (homozygosity or heterozygosity for the DQ2), resulted in this study.

These results were similar to those simultaneously reported by a recent survey that found a similar CD incidence at 3 years of age in children who received a daily dose of 100 mg of gluten between 16 and 24 weeks of age and the children who began receiving gluten at 24 weeks (1.23; 95% CI 0.79 to 1.91).<sup>13</sup> Likewise, the R generation study concluded that there was no difference in the development of CD autoimmunity whether gluten is introduced before or after 6 months of age.<sup>27</sup>

The results of both these studies are in agreement with the conclusions of the most recent paper, which reports the results of the multicentric TEDDY study. According to this study, neither the early (<17 weeks) nor the delayed introduction of gluten-containing cereals (>26 weeks) is a risk factor for the later development of CD-associated autoimmunity and biopsy-proven CD.<sup>16</sup>

On the contrary, the Norwegian study reported a slightly increased risk for children introduced to gluten when complementary feeding was started after 6 months of age (OR 1.27; 95% CI 1.01 to 1.65), but not for those receiving gluten before 4 months of age.<sup>26</sup> Only one paper described an increased risk of developing CD-related autoimmunity in two groups of children introduced to gluten earlier and later, respectively, than the reference period (4–7 months of age).<sup>15</sup> However, the significance for the group exposed to gluten after 7 months of age was very narrow, with a HR of 1.87 (95% CI 0.97 to 3.60), while the fivefold risk shown by children introduced to gluten before 3 months of age

**Table 1** Effect of BF and duration of BF on the development of CD

Reference	Number of infants/children enrolled	Length of observation	Effect of BF	Effect size	GRADE score	Bias risk
Retrospective studies						
Auricchio <i>et al</i> <sup>17</sup>	Biopsy-proven CD=216 controls=289	NA	BF >30 days protective	OR 4.05 (95% CI 2.2 to 7.2)	2	High
Greco <i>et al</i> <sup>18</sup>	Biopsy-proven CD=201 controls=1949	NA	BF <90 days protective	OR 4.97 (95% CI 3.5 to 6.9)	2	High
Fälth-Magnusson <i>et al</i> <sup>19</sup>	Biopsy-proven CD=72 controls=264	NA	BF >2.5 months protective	p<0.0002	2	High
Ascher <i>et al</i> <sup>20</sup>	85 (8 found to have silent CD at biopsy)	NA	No effect of duration of BF on CD development	6.5 months for cases vs 5.0 months for controls p ns	2	High
Peters <i>et al</i> <sup>23</sup>	Biopsy-proven CD=143 controls=137	NA	BF >2 months protective	OR 0.37 (95% CI 0.21 to 0.64)	1	High
Roberts <i>et al</i> <sup>25</sup>	Biopsy-proven CD=90 controls=248 521	NA	None	22 vs 38; p=0.28	1	High
Decker <i>et al</i> <sup>24</sup>	Biopsy-proven CD=157 controls=862	Up to a mean age of 13.9 years	None	OR 0.93 (95% CI 0.69 to 1.25)	1	High
Prospective studies						
Ivarsson <i>et al</i> <sup>21</sup>	Biopsy-proven CD=627 controls=1254	NA	Protective when CD diagnosis made in patients <2 years; none for CD diagnosis made in patients <2 years	p<0.001 p NS	2	High
Ziegler <i>et al</i> <sup>14</sup>	1610 (27 developed CD autoimmunity*)	From birth to 12 years of age	None	0–3 months OR 0.88 (95% CI 0.2 to 2.7) 3.1–6 months OR 1.2 (95% CI 0.4 to 3.6) >6 months OR 1.00 (reference)	3	Medium
Norris <i>et al</i> <sup>15</sup>	1560 (51 developed CD autoimmunity*)	From birth to 10 years of age	None	1.02 (95% CI 0.99 to 1.05)	3	Medium
Welander <i>et al</i> <sup>22</sup>	Biopsy-proven CD=44 controls=936	At 10 years of age	None	0–2 months OR 0.7 (95% CI 0.2 to 3.1) 3–4 months OR 0.7 (95% CI 0.2 to 3.2) 5–6 months OR 0.3 (95% CI 0.0 to 2.1) 7–8 months OR 1.4 (95% CI 0.7 to 3.1) 9–10 months OR 1.3 (95% CI 0.6 to 2.8) 11–12 OR 1.00 (reference)	3	High

Continued

**Table 1** Continued

Reference	Number of infants/children enrolled	Length of observation	Effect of BF	Effect size	GRADE score	Bias risk
Størdal <i>et al</i> <sup>26</sup>	Biopsy-proven CD=324 controls=81 834	From birth to 12 years of age	BF >1 year predisposing	<6 months reference 6–12 months OR 1.27 (95% CI 0.85 to 1.86) >13 months OR 1.49 (95% CI 1.01 to 2.21)	3	High
Jansen, 2014 <sup>27</sup>	1679 (43 developed CD autoimmunity*)	At 6 years of age	No difference in proportion of BF children in group who developed or do not have autoimmunity	p ns	3	Medium
Vriezinga <i>et al</i> <sup>13</sup>	944 (105 developed CD autoimmunity*, out of them 77 biopsy-proven overt CD)	From birth to 3 years of age	None	Overt CD 0 months OR 0.90 (95% CI 0.22 to 3.6) <3 months OR 1.3 (95% CI 0.41 to 4.1) 4–5 months OR 1.5 (95% CI 0.57 to 4.1) >6 months OR 1.2 (95% CI 0.67 to 2.2)	4	Low
Lionetti <i>et al</i> <sup>12</sup>	832 (117 developed CD autoimmunity*, out of them 86 biopsy-proven overt CD)	From birth to 10 years of age	None	CD autoimmunity OR=1.0 (95% CI 0.9 to 1.0) Overt CD OR=1 (95% CI 0.9 to 1.1)	4	Low

\*Serological positivity of tTG antibodies.  
BF, breast feeding; CD, celiac disease; NA, not available; NS, not significant; tTG, tissue transglutaminase.

**Table 2** Effect of BF at the time of gluten introduction on the development of CD

Reference	Number of infants/children enrolled	Effect of BF during gluten introduction	Effect size	GRADE score	Bias risk
Retrospective studies					
Fälth-Magnusson <i>et al</i> <sup>19</sup>	Biopsy-proven CD=72 controls=264	Protective	OR 0.35 (95% CI 0.17 to 0.66)	2	High
Ascher <i>et al</i> <sup>20</sup>	85 (8 found to have silent CD at biopsy)	None	NS	2	High
Peters <i>et al</i> <sup>23</sup>	Biopsy-proven CD=143 controls=137	Protective	OR 0.46 (95% CI 0.27 to 0.78)	1	High
Prospective studies					
Ivarsson <i>et al</i> <sup>21</sup>	Biopsy-proven CD=627 controls=1254	Protective	OR 0.55 (95% CI 0.4 to 0.77)	2	High
Norris <i>et al</i> <sup>15</sup>	1560 (51 developed CD autoimmunity*)	None	OR=1.32 (95% CI 0.76 to 2.28)	3	Medium
Størdal <i>et al</i> <sup>26</sup>	Biopsy-proven CD=324 controls=81 834	None	BF >1 months after introduction OR 1.17 (95% CI 0.74 to 1.87) BF <1 months after introduction OR 0.65 (95% CI 0.37 to 1.14)	3	High
Vriezinga <i>et al</i> <sup>13</sup>	944 (105 developed CD autoimmunity,* out of them 77 biopsy-proven overt CD)	None	OR=1.35 (95% CI 0.57 to 4.1)	4	Low
Lionetti <i>et al</i> <sup>12</sup>	832 (117 developed CD autoimmunity,* out of them 86 biopsy-proven overt CD)	One	CD autoimmunity OR=1.5 (95% CI 0.77 to 3.0) Overt CD OR=1 (95% CI 0.6 to 2.3)	4	Low
Aronsson <i>et al</i> <sup>16</sup>	6434 (773 developed autoimmunity,* 307 overt biopsy-proven CD)	None	Overt CD OR=1.13 (95% CI 0.88 to 1.46)	3	Medium

\*Outcome of CD autoimmunity has been considered the serological positivity of tTG antibodies.  
BF, breast feeding; CD, celiac disease; NS, not significant; tTG, tissue transglutaminase.

had more robust statistical significance (HR 5.17, 95% CI 1.44 to 18.57).

### Amount of gluten at weaning and development of CD

None of the papers included in our analysis compared the effect of different amount of gluten given to children during weaning on the risk of CD. This aspect is mostly unknown and the scant available data come from a Swedish experience. However, papers reporting the paradigmatic Swedish experience were excluded from the analysis, since negative controls were not included in the description.<sup>28</sup>

## DISCUSSION

### Breast feeding and CD

Our review shows that some studies reported a protective effect of breast feeding on the risk of developing CD while others, on the contrary, reported no effect. The latter are the most recent, and have the highest GRADE

score and the lowest bias risk. Accordingly, all the prospective studies included in our analysis, except one, concluded that the duration of breast feeding (exclusive and/or complementary) and/or gluten introduction while the infant is still breast fed had no impact on the risk of developing CD. In addition, the only prospective study to describe a protective effect of breast feeding, reported this effect only in the group of children diagnosed with CD below 2 years of age and not for those diagnosed over this age. These conclusions contrasted with the meta-analysis by Akobeng *et al*,<sup>29</sup> which reported protection against CD with longer duration of breast feeding, based on the findings from five earlier retrospective studies (OR 0.48; 95% CI 0.40 to 0.59). So, the limited effect of breast feeding found may only represent a delay of the development of the symptoms and clinical signs of CD, rather than being a real prevention of this condition.

This result is quite surprising, since earlier studies and reviews indicated a potential protective effect on CD

**Table 3** Effect of the time of gluten introduction on the development of CD

Reference	Age of gluten introduction	Effect size	GRADE score	Bias risk
Retrospective studies				
Fälth-Magnusson <i>et al</i> <sup>19</sup>	Mean age at gluten introduction: 6-month for CD cases 6.1 for controls	Range 4–7 Range 4–10 p NS	2	High
Peters <i>et al</i> <sup>23</sup>	Continuous risk per month (1–12 months) <3 vs >3 months 3 vs 4 months 4 vs 5 months >5 months	HR 0.98 (95% CI 0.86 to 1.11) HR 0.72 (95% CI 0.29 to 1.79) HR 0.52 (95% CI 0.18 to 1.44) HR 1.21 (95% CI 0.40 to 3.68) HR 0.72 (95% CI 0.28 to 1.85)	1	High
Prospective studies				
Ivarsson <i>et al</i> <sup>21</sup>	1–4 months 5–6 months (reference) 7–12 months	HR 1.4 (95% CI 0.87 to 2.4) HR 0.76 (95% CI 0.41 to 1.4)	2	High
Ziegler <i>et al</i> <sup>14</sup>	<3 months 3–6 months (reference) >6 months	HR 2.3 (95% CI 0.3 to 18.2) HR 0.7 (95% CI 0.3 to 1.8)	3	Medium
Norris <i>et al</i> <sup>15</sup>	1–3 months 3–4 months (reference) >7 months	HR 5.17 (95% CI 1.44 to 18.57) HR 1.87 (95% CI 0.97 to 3.60)	3	Medium
Welander <i>et al</i> <sup>22</sup>	3–4 months 5–6 months (reference) 7–8 months	HR 1.0 (95% CI 0.3 to 3.3) HR 1.1 (95% CI 0.6 to 2.0)	3	High
Størdal <i>et al</i> <sup>26</sup>	<4 months 5–6 months (reference) >6 months	OR 1.27 (95% CI 1.01 to 1.65) OR 1.05 (95% CI 0.69 to 1.58)	3	High
Jansen <i>et al</i> <sup>27</sup>	>6 months	NS	3	Medium
Vriezinga <i>et al</i> <sup>13</sup>	16–24 weeks	HR 1.23 (95% CI 0.79 to 1.91)	4	Low
Lionetti <i>et al</i> <sup>12</sup>	6 vs 12 months	HR 0.9 (95% CI 0.6 to 1.4)	4	Low
Aronsson <i>et al</i> <sup>16</sup>	<17 weeks 17–26 (reference) >26 weeks	HR 0.59 (95% CI 0.33 to 1.04) HR 0.90 (95% CI 0.69 to 1.18)	3	Medium

CD, celiac disease; NS, not significant.

onset.<sup>30</sup> Indeed, breast milk may independently prevent intestinal infections, which are thought to be one of the triggering factors for CD, modulate the intestinal microbiota, increasing the number of bifidobacteria, and boost the mechanisms of oral tolerance by means of several immunomodulatory molecules, offering a high biological plausibility to the interpretation of a protective effect on immune-mediated diseases such as CD. No studies are, at the moment, available to explain the lack of a protective role of breast feeding on the risk of CD. A recent study, however, revealed that breast milk of mothers with CD has reduced concentrations of immunoprotective compounds (tumour growth factor (TGF)- $\beta$ 1 and sIgA) and bifidobacteria 16S rRNA gene copy numbers as compared with breast milk of healthy mothers, which could presumably diminish the protective effects of breast feeding on the child's future risk of developing CD.<sup>31</sup>

A recent prospective study in a cohort of infants at family risk of CD has shown that the HLA-DQ2/8 genotype may independently contribute to influencing the

composition of gut microbiota.<sup>7 32</sup> In this regard, the studies investigating the role of breast feeding on CD development have included different control populations with heterogeneous genetic backgrounds, thereby representing a non-controlled variable in most of them. Additional environmental factors that could confound the potential role of breast feeding on CD, directly or via gut microbiota modulation, include mode of delivery, incidence of infections and maternal diet.<sup>5 33 34</sup> These pieces of evidence suggest that a number of host and environmental factors, besides gluten intake, might play a relevant role in the onset of overt CD, thus confounding the statistical analysis on the effect of breast feeding.

### Time of gluten introduction and CD

It is quite clear from our analysis that the age of children at exposure to gluten during the weaning process bears no effect on CD development. Only two papers found a correlation between the time of gluten introduction and development of CD. Norris *et al*<sup>15</sup> found an increased risk for both early and late gluten

introduction while Strødal *et al*<sup>26</sup> reported an increased risk for CD when gluten is introduced after 6 months of age. It is noteworthy that these indices of risk are very mild, with a large variation and a possible role of further residual confounders, therefore showing only a low statistical significance. Both the two recent large, prospective studies demonstrated, with a very solid intervention design, high GRADE score and low bias risk, that neither early (4 months of age) nor late (1 year of age) introduction of gluten impacts the later development of CD, respectively.<sup>12 13</sup> It is noteworthy that, in the Italian multicentre study, the group of baby girls (but not boys) at high genetic risk of CD, carrying the DQ2 haplotypes in homozygosis, who were introduced to gluten earlier (at 6 months)<sup>12</sup> had a higher prevalence of CD even at 5 years of age. Similarly, in the multicentre European trial,<sup>13</sup> the girls (and again, not the boys) in the group where gluten was introduced early (at 4 months) had a higher prevalence of CD (21%) at 5 years of age than those who were first exposed to gluten at 6 months (8.5%).

Also considering studies on food allergy, one study (GINIplus and LISApplus) reported that a delayed introduction of solid foods or the avoidance of highly allergenic foods during the first year does not seem to be beneficial for allergy prevention, while only a very early (before week 17 of age) introduction of solids may increase the risk of later manifestations of eczema.<sup>35</sup> Although CD has an obviously different pathogenetic mechanism with respect to eczema, most epidemiological studies support the hypothesis that, after the fourth month of age, any solid can be safely introduced, without increasing the risk of developing reactions to food antigens.<sup>36</sup> Accordingly, there is no reason to delay the first exposure to any solid food, including foods considered to be highly allergenic. Theoretically, the immunodevelopmental processes and the generation of regulatory T-cells and cytokines driving oral tolerance are influenced by the structure of the microbiota colonising the newborn intestine, which in turn evolves in time mainly influenced by dietary changes, particularly cessation of breast feeding and the introduction of solids.<sup>37 38</sup> Following these considerations, the timing of gluten introduction suggested by the current ESPGHAN commentary on complementary feeding appears outdated and is no longer evidence-supported, since it was drafted before the publication of the studies reviewed here.<sup>39</sup>

The Swedish observations on the celiac epidemic in the late 80s, which occurred when the amount of gluten given to infants during weaning was dramatically increased, suggest that the amount of gluten itself might have a key role.<sup>28</sup> However, none of the studies suitable for inclusion into a systematic review have collected information about the load of gluten during the early feeding phases. It is likely that the amount of gluten introduced at weaning might play a pivotal role in triggering CD in predisposed children. Information about

this issue might provide us an important clue to understand how early feeding practices might influence CD development. Studies about the role of varying amounts of gluten given to infants during weaning are not available. Even observations reporting the Swedish epidemic of CD that occurred after changes in infant feeding practices fail to provide information about the actual amount of gluten that was introduced to the involved infants during weaning.

The generalisability of our results has been enhanced by the involvement of children from both Europe and the USA, and the uniformity of the diagnosis of CD, made by means of the serum positive titre of anti-tTG antibodies and duodenal biopsy.

On the other hand, most of the papers included in our analysis have a high bias risk. Several papers included controls from general populations not selected for at-risk genetic background and others enrolled children at genetic risk for T1DM, a condition partially sharing the same type of genetic predisposition as CD. Within these limits, all the studies that enrolled DQ2+children, with a prospective design, are in agreement that both breast feeding and the timing of gluten introduction during weaning do not impact on the development of CD.

The results of this systematic review are consistent with those of a recent meta-analysis by Szajewska *et al*,<sup>40</sup> which has included the same studies. With respect to that review, we scored the papers according to their bias risk and discussed the results from a different angle, including the data that support a role for additional variables in the development of CD, such as differences in microbiota and breast milk composition. On the contrary, the paper by Szajewska *et al* is focused exclusively on the role of gluten introduction into the diet.

In conclusion, there is currently no evidence to recommend avoiding either an early (at 4 months of age) or a late (at or after 6 or even 12 months) gluten introduction in children at risk of CD. The possible exception of DQ2 homozygous girls,<sup>12 13</sup> where an early introduction of gluten appears to be associated with a greater risk of subsequent development of CD must, however, be acknowledged, and requires further study, possibly representing an early manifestation of 'medicine of gender'. Accordingly, no specific general recommendations about gluten introduction or optimal breastfeeding duration can be presently provided on evidence-based criteria.

Even in the absence of evidence of the protective effect of breast feeding, it must be reiterated that breast feeding should be implemented whenever possible in all infants, including those at genetic risk for CD, for its many, well-documented benefits, including its unique role in maternal–infant bonding.

Further studies that include variables so far neglected are needed to progress in the identification of critical factors and predictive models of CD development.

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