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Systematic review: early infant feeding and the prevention of coeliac disease

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SUMMARY

Background
PREVENTCD, Prevent Coeliac Disease, is an international project investigating the hypothesis of possible induction of tolerance to gluten in genetically predisposed children through introducing small quantities of gluten during the period of breastfeeding.

Aim
To summarise current knowledge on the possible relationship between early feeding practices and the risk of coeliac disease (CD).

Methods
The Cochrane Library, MEDLINE, and EMBASE databases were searched in May 2011, and the search was updated in January 2012, and again in July 2012.

Results
Breastfeeding (BF) and CD: some studies show a protective effect of BF, while others show no effect. No studies have shown a long-term preventive effect. *BF at the time of gluten introduction and CD*: Results from a meta-analysis of five observational case-control studies suggest that BF at gluten introduction is associated with a lower risk of CD compared with formula feeding. It is unclear whether BF provides a permanent protection or only delays the onset of CD. *Timing of gluten introduction*: The data suggest that both early (≤ 4 months) and late (≥ 7 months) introduction of gluten may increase the risk of CD. *Amount of gluten at weaning (and later) and CD*: One incident case-referent study documented that the introduction of gluten in large amounts compared with small or medium amounts increased the risk of CD.

Conclusions
In the absence of clear evidence, in order to decrease the risk of later coeliac disease, it is reasonable to avoid both early (<4 months) and late (≥ 7 months) introduction of gluten, and to introduce gluten while the infant is still being breastfed. Future studies may clarify the remaining uncertainties.

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INTRODUCTION

Coeliac disease (CD) has been recently defined by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) as 'an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy. CD-specific antibodies comprise autoantibodies against TG2 (anti-TG2), including endomysial antibodies (EMA), and antibodies against deamidated forms of gliadin peptides (DGP)'.[1]

The incidence of CD is as high as 0.5–1.6% in the general population in Europe and North America.[2] Higher rates are reported in first-degree relatives of patients with CD, patients with autoimmune diseases such as type 1 diabetes or autoimmune thyroid disease, patients with some chromosomal aberration disorders (e.g. Down syndrome, Turner syndrome, Williams syndrome), and patients with selective IgA deficiency. The course of CD may be symptomatic with the occurrence of gastrointestinal and nongastrointestinal symptoms. However, CD also may develop as an asymptomatic disease.[1] A lifelong, gluten-free diet introduced only when a conclusive diagnosis has been made is the recommended treatment.

Recently, key stakeholders representing a wide range of knowledge related to CD concluded that the option of primary prevention should be fully explored, which requires combined epidemiological, clinical and basic scientific research efforts. In particular, a great deal of attention should be focused on the relationship between early nutrition and later development of CD, particularly on the timing and circumstances of gluten introduction.[3]

PREVENTCD, Prevent Coeliac Disease (http://www.preventcd.com), is an international project, sponsored by the European Union 6th Framework Programme. The aim of this project is to investigate the hypothesis of possible induction of tolerance to gluten in genetically predisposed children through the introduction of small quantities of gluten during the period of breastfeeding. The pivotal objective of the project is to significantly reduce the number of people suffering from CD in Europe by developing primary prevention strategies. To achieve this, PREVENTCD involved the following areas of research in relation to CD development: (i) infant feeding, especially breastfeeding and gluten introduction (based on a randomised, double-blind, controlled trial involving high-risk infants and the Food Frequency Questionnaire as well as a Swedish CD screening study among 12-year-old children from two population cohorts that differ with respect to infant feeding); (ii) immunological response to gluten introduction; and (iii) genetic factors (both HLA and non-HLA alleles). A detailed description of each study field has been published separately.[4–7] Revision of the current European guidelines for early nutrition to prevent CD is the final objective of PREVENTCD. This, however, can only be achieved when all data are analysed. The analysis of all data will be feasible only after 2013 when all infants recruited into the intervention study will have reached 3 years of age, the code will have been broken, and thus, the study unblinded.

The purpose of this report developed by PREVENTCD investigators is to summarise current knowledge concerning the possible relationship between early feeding practices and the risk of developing CD. In particular, a systematic review was designed to answer the following clinical questions grouped into four categories important for making future recommendations:

(i) Breastfeeding (BF) and CD (Does any BF reduce the risk of developing CD in early childhood? Is there a difference between any or exclusive BF in regard to risk reduction? Is the duration of BF related to the risk of developing CD?).

(ii) BF at the time of gluten introduction and CD (Is gluten consumption while being breastfed important for risk reduction?).

(iii) Timing of gluten introduction (Is age of gluten introduction important to the risk of developing CD?).

(iv) Amount of gluten at weaning (and later) and CD (Is the amount of gluten ingested an independent risk factor for the development of CD in early childhood? Is there a threshold level of gluten consumption for developing CD in early childhood?).

In addition, we analysed whether manipulation of the intestinal microbiota through the administration of microbial supplements (probiotics) and/or substrates (prebiotics) has an effect on the risk of CD. This was based on recent studies suggesting that aberrant development and maturity of the gut microbiota is among the environmental factors to be associated with CD.[8–11]

An update of this systematic review together with an update of current recommendations is planned immediately after findings from PREVENTCD are available.

MATERIALS AND METHODS

The systematic review of the literature was initially performed in May 2011 and was updated in January 2012, and again in July 2012. The electronic searches were based on the content of the Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library),

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MEDLINE and EMBASE. Several searches were performed separately for all categories of clinical questions listed above. In addition, the reference lists from identified studies and key review articles were searched. Researchers working in the field, primarily partners in PREVENTCD, were contacted for any unpublished data. Certain publication types (i.e. letters to the editor, abstracts, proceedings from scientific meetings) were excluded, unless a full set of data was available from the authors. No language restriction was imposed. The search was carried out independently by two reviewers (AC, MP). The most recent update was carried out by one reviewer (HS). The following search terms were used separately for each clinical question:

(i) celiac or coeliac or CD or sprue or gluten enteropathy.
(ii) breast-feeding or breastfeeding or breast feeding or breastfed.
(iii) child or childhood or children or child* or infant* or toddler or early.
(iv) gluten and (timing or time) and introduction.
(v) amount or quantity.
(vi) probiotic* or prebiotic*.

Types of studies

Studies of any design, preferentially randomised controlled trials (RCTs), investigating the potential association between early feeding practices and risk of CD were eligible for inclusion. In addition, previously published systematic reviews/meta-analyses were considered for inclusion.

Types of participants

Participants involved in the prospective studies had to be infants at population risk or increased risk of developing CD (defined by HLA status, first-degree relative with CD or type 1 diabetes mellitus). For retrospective studies, participants had to be children or adults with CD diagnosed by small bowel biopsy or presenting with positive serology indicative of CD.

Types of intervention (interventional studies)

Interventions used had to be a gluten-containing product meeting any definition (e.g. cereals, flour or any other foods containing gluten, preparations manufactured for research purposes). In addition, studies that assessed the effect of probiotics and/or prebiotics compared with control (placebo or no treatment) were considered.

Outcome measures

The primary outcome measure was CD or the development of CD-associated autoantibodies (i.e. anti-TG2 or EMA). The studies should have assessed the risk of CD in people who were:

(i) Ever breastfed compared with those never breastfed.
(ii) Exclusively breastfed compared with those receiving any human milk.
(iii) Breastfed for different periods of time (short compared with long breastfeeding according to the definition given by the authors).
(iv) Breastfed at the time of gluten introduction compared with those who were not.
(v) Given gluten for the first time at different ages (early compared with late introduction according to the definition given by the authors).
(vi) Given gluten in different amounts (by any quantity units or thresholds used by the authors).

Data collection and analysis

An initial screening of the title, abstract and keywords of every record identified was performed. The next step was retrieval of the full text of potentially relevant trials. Two reviewers (AC, MP) 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 (HS).

Assessment of methodology of included studies

The reviewers independently, but without being blinded to the authors or journal, assessed the risk of bias in the studies that met the inclusion criteria. For interventional studies, The Cochrane Collaboration's tool for assessing risk of bias was used, which includes the following criteria: adequacy of sequence generation, allocation concealment and blinding of participants, personnel and outcome assessors; incomplete outcome data are addressed, free of selective outcome reporting and free of other sources of bias.[12] There is no tool for assessing the quality of nonrandomised trials that would be widely recognised as most effective.[13] Again, we chose to use The Cochrane Handbook for Systematic Reviews of Interventions.[14]

Statistical methods

Although a meta-analysis of the available data was initially planned, after data collection it turned out not to be feasible for any of the outcomes. The reason was the different definitions of outcomes of interest used by different authors. For example, breastfeeding was defined as the duration of breastfeeding by some authors or time

intervals when it was ceased by others. In addition, the timing of gluten introduction was reported as either a point in time or a certain time interval. Whenever possible, we report the binary measure for individual studies as the odds ratio (OR) between the experimental and control groups with 95% CI or as the hazard ratio (HR), as presented by the authors of individual trials. Continuous outcomes are presented as the mean with standard deviation (s.d.) or the median with ranges, again as reported by the authors. For outcomes of interest that have previously been reviewed systematically, we have summarised the findings from those reviews.

RESULTS

Description of studies included in the review

Twenty-eight potentially eligible studies were initially identified. During a repeat search (July 2012), one additional study was identified. Eventually, 12 studies were included, the characteristics of which are summarised in Table S1.[8, 15–25] Eleven included trials were of observational design. Two studies[21, 25] used healthy children as controls. In those two studies, CD, based on positive serology but not biopsy-proven CD, was assessed. Three of the studies were cohort studies. Studies by Norris *et al.*[21] and Ziegler *et al.*[25] followed children at genetic risk of CD or type 1 diabetes. Welander *et al.*[24] performed a population-based cohort study. Only one interventional study was identified.[8] This study recruited 34 infants at risk of CD (positive for HLA DQ2 and/or HLA DQ8). The families of four of these infants refused to participate. Therefore, from 6 to 12 months of age, 30 infants were randomly assigned to receive either a gluten-free diet (delayed exposure group, $n = 13$) or a gluten-containing diet from 6 months of age (early exposure group, $n = 17$). In all infants, a normal, gluten-containing diet was administered at 12 months. While the study was reported as a randomised, double-blinded trial, it lacked adequate information to assess the overall risk of bias (unclear randomisation, allocation concealment and blinding). The researchers assessed CD development and serological evaluation. In addition, the stool samples of eight randomly selected infants in each group were collected for microbiota and metabolome analyses from day 7 to 24 months of age.

The protocol of one ongoing multicenter, randomised, double-blind, placebo-controlled study exploring the role of early infant feeding on CD development (PREVENTCD) has been published.[4]

In addition, two systematic reviews were identified. Akobeng *et al.*[26] conducted a systematic review of the literature that explored the effect of BF compared with no BF, the effect of the duration of BF and the effect of BF at the time of the introduction of dietary gluten. The MEDLINE, EMBASE and CINAHL databases were searched (until May 2004) as well as reference lists. No language restrictions were applied. An attempt to identify unpublished data was made. Two reviewers independently assessed the methodological quality of the included trials using the Critical Appraisal Skills Programme tool for case-control studies. Studies were assigned an overall rating of A (low risk of bias), B (moderate risk of bias) or C (high risk of bias). A total of six case-control studies,[15, 16, 18–20, 22] all of which were also identified by us, were included. All included studies were graded B. To assess the effect of BF at the time of gluten introduction, a meta-analysis of all included case-control studies was performed using a fixed effect model. For other outcomes, a meta-analysis was not feasible, so only a systematic review was performed.

The second systematic review by Nash *et al.*[27] was aimed at determining if exclusive BF reduced the risk of CD. The MEDLINE, EMBASE and CINAHL, databases were searched (presumably in 2003) for cohort studies and case-control studies, if published in English and available in the library of the reviewer. No attempt was made to identify unpublished data. The methodological quality of included trials was described, although not formally assessed. Three case-control trials[16, 19, 22] were included in the review.

For the current review, 16 publications were excluded (Table S2). Among them, four studies were of retrospective design with no control group, 10 were reviews and one was a letter without a description of the methodology provided. In addition, one trial was not included because it explored the changing practices of early infant feeding in relation to the incidence of CD.

A summary of the results for all clinical questions is presented in Tables 1–3.

Breastfeeding and coeliac disease

Exclusive breastfeeding vs. any breastfeeding. One systematic review[27] assessed the possible relationship between exclusive BF and a reduction in the risk of CD. Three case-control studies[16, 19, 22] were included in the review ($n = 2935$; 560 cases and 2375 controls). All of the studies were retrospective and open to recall bias. There was no evidence suggesting that exclusive BF compared with formula or mixed feeding either reduces the risk of CD or delays the onset of symptoms.

Table 1 | Duration of breastfeeding and coeliac disease

	Reference	*N*	Duration of BF	Effect size	Effect
Studies included in the systematic review by Akobeng *et al.*[26]	Auricchio 1983[16]	505	Breastfed <30 days or bottle-fed has higher risk of CD than breastfed >30 days	OR 4.05 (2.2–7.27)	Short BF predisposing
	Ascher 1997[15]	81	BF in cases vs. controls: 6.5 (range 1.5–9) months vs. 5 (0–14) months	N.S.	No effect
	Falth-Magnusson 1996[18]	336	Median BF duration: 2.5 months (CD) vs. 4 months (control)	$P < 0.003$	Short BF predisposing
	Greco 1988[19]	2150	BF <90 days 5 times more likely to develop CD	OR 4.97 (3.5–6.9)	Short BF predisposing
	Ivarsson 2002[20]	1272	Children <2 years: median BF duration 5 months for CD vs. 7 months for controls	$P < 0.001$	Short BF predisposing
			Children >2 years	N.S.	No effect
	Peters 2001[22]	280	Risk of developing CD decreased by 63% for children BF >2 months vs. BF <2 months	OR 0.37 (0.21–0.64)	Short BF predisposing
Decker 2010[17]		157 cases + 862 controls	The rate of BF in patients with CD (86.6%) was higher compared with control subjects (76.5%)	OR 1.99 (1.12–3.51).	No effect
			The average duration of BF – 5.18 months (CD) vs. 5.25 months (controls)	N.S.	
Norris 2005[21]		1560 (51 developed autoimmunity)	No protective effect of breastfeeding. BF duration in CD autoimmunity-positive children was 8.3 (8.8) months and BF duration in CD autoimmunity-negative children was 6.7 (6.8) months	OR 1.02 (0.99–1.05)	No effect
Roberts 2008[23]		248 521 (cases $n = 90$)	No significant association between CD and BF	N.S.	No effect
Welander 2010[24]		Cases $n = 44$/controls $n = 9364$	No associations between breastfeeding duration, age at gluten introduction, and future CD (biopsy verified)	N.S.	No effect
Ziegler 2003[25]		1610 (27 developed autoimmunity)	No trend in antibodies to tissue transglutaminase C was observed for the duration of BF.	N.S.	No effect

Table 2 | Breastfeeding at the time of gluten introduction

Reference	Design		OR	Effect	Strengths/Limitations
Akobeng 2006[26]	Meta-analysis of case-control studies	Ascher	1.54 (0.27–10.56)	No effect	Not clear whether BF provides long-term protection or just delays the symptoms.*
		Falth-Magnusson	0.35 (0.17–0.66)	Protective	
		Ivarsson	0.5 (0.4–0.64)	Protective	
		Peters	0.46 (0.27–0.78)	Protective	
		Pooled	0.48 (0.4–0.59)	Protective	
Norris 2005[21]	Prospective observational study		HR 1.32 (0.76–2.28)	No effect	Prospective design; however, small number of subjects in whom the outcome measures occurred; use of CD autoimmunity as a surrogate for biopsy-diagnosed CD.

* Comment applies to all studies listed under Akobeng 2006.

Ever breastfed vs. never breastfed. Two studies reported this exposure.[17, 22] In the study by Decker *et al.*,[17] more children with CD were ever breastfed (OR 1.99, 95% CI 1.12–3.51; $P = 0.015$).[17] Peters *et al.*[22] reported a lower risk of CD in ever-breastfed children compared with those who were never breastfed. Moreover, the longer the breastfeeding duration compared with no breastfeeding at all, the lower the risk of CD.

Duration of breastfeeding and coeliac disease. Eleven studies[15–25] evaluated the relationship between the duration of BF and CD (Table 1). Data from six of these trials[15, 16, 18–20, 22] were analysed in the systematic review by Akobeng *et al.*[26] Based on the findings from five of the studies, the reviewers stated that protection against CD with longer duration of BF was reported. However, more current evidence did not show that short-term BF was associated with an increased risk for CD.[17, 21, 23–25] With few exceptions (e.g. Welander[24]), most of the data were collected retrospectively.

Breastfeeding at the time of gluten introduction and coeliac disease

Five studies[15, 18, 20–22] explored the role of BF at the time when gluten-containing products were introduced into the infants' diet (Table 2). Three of them reported a significantly reduced risk of CD in children who were breastfed when they started receiving gluten. In one small study[15] and in one large prospective trial,[21] no statistically significant difference was observed between the case and control groups. In addition to the prospective design, one additional feature of the study by Norris[21] that differentiates it from earlier studies is that it focused on children at high risk for CD (in a cohort originally designed to study children at high risk of developing type 1 diabetes). All studies but the latter study[21] were considered in the meta-analysis by Akobeng *et al.*[26] In the latter meta-analysis, the pooled risk for developing CD in children breastfed compared with those who were not breastfed at the time of gluten introduction was reduced by almost 50% (OR 0.48; 95% CI 0.40–0.59).

Timing of gluten introduction

Six studies[18, 20–22, 24, 25] explored whether the timing of the first introduction of gluten-containing products may influence the risk of CD (Table 3). Most of these defined the moment of gluten introduction as a time interval (e.g. <3 months or 7–8 months). Therefore, the definitions used by the authors were different, thus, results were difficult to compare.

Out of five trials, two[21, 22] reported a significantly increased risk of developing CD (or CD-associated autoantibodies) related to the timing of gluten introduction. The prospective, observational, cohort study by Norris *et al.*[21] revealed that both early (less than 3 months) and late (more than 7 months of age) introduction of gluten to children at increased risk of CD and type 1 diabetes mellitus was associated with an increased risk of CD. Children exposed to gluten before 3 months of age had a five-fold higher risk of developing CD than those with gluten introduced between 4 and 6 months of age. The risk was slightly higher when gluten was first given at the age of 7 months or later compared with when it was first given at 4–6 months of age (see Table 3 for details). The strength of this study is its prospective design; however, it

Table 3 | Time of gluten introduction

Reference	Age	Measure	Effect size	Effect	Limitations
Falth-Magnusson 1996[18]	Age of gluten introduction in CD infants 6 months (mean: 6; range: 4–7) compared with 6.1 months (6; 4–10) for reference infants.			No effect	
Ivarsson 2002[20]	1–4 months	Adjusted OR for breastfeeding and gluten introduction	1	Reference	Introduction between 5–6 months dominated (CD cases 82% and referents 73%), thus the other groups are the ones that cause uncertainty*
	5–6 months		1.4 (0.87–2.4)	No effect	
	7–12 months		0.76 (0.41–1.4)	No effect	
Norris 2005[21]	1–3 months	Unadjusted HR	2.94 (0.83–10.4)	Predisposing	Small number of subjects in whom the outcome measure occurred; the use of CD autoantibodies as a surrogate for biopsy-diagnosed CD; the amount of gluten during introduction not assessed. Time of gluten introduction in reference population quite young; most EU countries at that time advised >6 months*
	4–6 months		1.0 (reference)	Reference	
	≥ 7 months		1.78 (0.92–3.42)	Predisposing	
Peters 2001[22]	>3 months vs. ≤ 3 months	Adjusted OR	0.72 (0.29–1.79)	No effect	
	≤ 3 months		1.0	No effect	
	>3 to ≤ 4 months		0.52 (0.18–1.44)	No effect	
	>4 to ≤ 5 months		1.21 (0.4–3.68)	No effect	
	>5 months		0.72 (0.28–1.85)	No effect	
Welander 2010[24]	0–2 months	Unadjusted HR	Not estimated	–	
	3–4 months		1.0 (0.3–3.3)	No effect	
	5–6 months		1.0 (reference)	Reference	
	7–8 months		1.1 (0.6–2.0)	No effect	
	9–10 months		Not estimated	–	
	11–12 months		Not estimated	–	
Ziegler 2003[25]	≤ 3 months	Unadjusted HR	2.3 (0.3–18.2)	No effect	Not CD, but the development of CD-associated autoantibodies was the outcome*
	3.1–6 months		1	Reference	
	>6 months		0.7 (0.3–1.8)	No effect	
	Unknown		1.0 (0.3–2.6)	No effect	

* Comment applies to all age groups.

has several limitations (e.g. the small number of subjects in whom the outcome measure occurred, the use of CD autoantibodies as a surrogate for biopsy-diagnosed CD). Moreover, the amount of gluten given during introduction was not assessed, thus, remains as a potential confounder. According to Peters *et al.*[22], no difference was found in the risk of CD depending on the time of gluten introduction for the majority of time intervals they defined. However, when adjusted for age, sex, number of inhabitants of residence and family predisposition to CD, the OR for >4 months vs. $\leq$4 months was 0.66 (95% CI 0.44–1.00). The remaining studies did not show a relationship between the timing of gluten introduction and the risk of developing CD.

In the only included RCT,[8] the researchers reported data on CD development and serological evaluation in a subgroup of eight infants in each study group. At 24 months, no significant difference was found in CD development, defined by the appearance of CD anti-tissue transglutaminase antibodies, onset of CD-related symptoms and/or evidence of autoimmune enteropathy, in the delayed exposure to gluten group compared with the early exposure to gluten group (0/8 vs. 1/8, respectively, relative risk 0.33, 95% CI 0.02–7.1). Similarly, there was no difference in anti-gliadin antibodies of the class IgG between groups except at 12 mo when the difference between the delayed exposure to gluten group compared with the early exposure to gluten group was of borderline statistical significance (0/12 vs. 8/13, relative risk 0.06 (95% CI 0.00–0.99).

Amount of gluten at weaning (and later) and CD

Only one study[20] analysed the amount of gluten that children received. In children younger than 2 years of age, the risk of developing CD was greater when gluten was introduced into the diet in large amounts than when introduced in small or medium amounts (adjusted OR 1.5, 95% CI 1.1–2.1). In older children, there was no effect.

Administration of probiotics and/or prebiotics

No studies that have addressed these issues were identified.

DISCUSSION

Breastfeeding and CD

There are studies that show a protective effect of breastfeeding as well as studies that show no effect. No studies have shown a long-term preventive effect of BF. Thus, whether or not BF protects or delays the clinical presentation of CD remains controversial. Despite the fact that there is controversy in the literature, this does not mean that breastfeeding does not have significant effects in preventing CD. Rather, this is more likely a reflection of the methodological inadequacy of investigating breastfeeding in ways that take into account all the complexity of interactions. The methodological problems likely to contribute to inconsistent results include first, the inability to randomise and blind. In general, the studies on breastfeeding are nonrandomised, retrospective or observational in design and, thus, produce inconclusive results. Second, the retrospective design of many studies addressing the association between breastfeeding and CD and the potential for parental recall bias impose methodological challenges. One may overcome the problem of parental recall bias by obtaining prospective feeding histories. Third, most of the studies that have examined the effect of breastfeeding on CD were carried out in unselected birth cohorts with regard to CD risk. Only a limited number of studies have assessed the effect of breastfeeding in high-risk infants. Inconsistencies may be also due to imprecise definitions of the intervention. Fourth, many studies do not make the distinction between 'exclusive breastfeeding' and 'any breastfeeding'. Finally, ideally, the diagnosis of CD should be based on widely agreed-upon criteria. However, in some of the studies on the effect of breastfeeding, CD-specific serology, not biopsy-proven CD, was assessed, making comparisons between the studies difficult.

The exact mechanisms that underlie the relationship between breastfeeding and possible protection against CD remain uncertain. Likely explanations have been extensively discussed in earlier studies and reviews.[28] In brief, it has been postulated that breast milk contains factors such as secretory IgA antibodies, lactoferrin, lysozyme and others that contribute to passive immunity. These factors may contribute to the reduced number of gastrointestinal infections potentially contributing to the pathogenesis of CD by increasing gut permeability or alterations to the immune system.[29] Moreover, human milk contains cytokines such as down-regulatory transforming growth factor β that may influence immune development and the type of immune response. When studying the interaction between breastfeeding and CD, the complex interactions between intestinal immunology, gut microbiome, genetic predisposition, gluten consumption and breastfeeding should be considered. In addition, human milk contains gluten in small quantities[30, 31]; this can perhaps induce tolerance to gluten as it has been suggested for other antigens.[32] Future studies are needed to fully understand the relationship between BF and CD.

BF at the time of gluten introduction and CD

Results from a meta-analysis of five observational case-control studies suggest that BF at the time of gluten introduction is associated with a lower risk of CD compared with formula feeding. However, the majority of these studies were based on retrospectively collected feeding data. It is unclear whether BF provides a permanent protection or only delays the onset of CD. Available data are insufficient to prove causality. Moreover, one more recent prospective study found no effect of BF at the time of gluten introduction on CD autoimmunity, but the effect on biopsy-proven CD is unknown.[21]

Timing of gluten introduction

The role of age at gluten introduction with respect to the risk of CD is unclear. The data from observational studies suggest that early ($\leq$3 months after birth), and possibly late ($\geq$7 months after birth), introduction of gluten may be associated with an increased risk of CD and probably should be avoided. The only interventional study suggested that delayed introduction of gluten (12 months of age) may be beneficial. However, the results of this RCT should be viewed with caution given the small sample size and unclear risk of bias.

Amount of gluten at weaning (and later) and CD

The results of one incident case-referent study documented that the introduction of gluten in large amounts compared with small or medium amounts increased the risk of CD.[20] These data support previous findings from the same country, i.e. Sweden. In the mid 1980s, this country experienced an epidemic of CD in children younger than 2 years of age. A twofold increase in the average daily consumption of gluten was followed by a fourfold rise in the incidence of CD. When gluten consumption decreased 10 years later, an abrupt fall in the incidence of CD was observed.[6] However, also the recommended age for gluten introduction was changed preceding both the start of the epidemic (from 4 to 6 months) and the end (back to 4 months), which changed the proportion of infants introduced to gluten while being breastfed. Still, the amount of gluten is likely to be a contributing risk factor for CD. Whether this is a dose–response or a threshold effect remains unknown. However, more recently, a quantitative model of CD development was suggested and an HLA-DQ2 gene dose effect in the development of CD was proposed.[33]An interaction between HLA-DQ2 expression and the available number of T-cell stimulatory gluten peptides was documented. In particular, the strongly increased risk of CD development for HLA-DQ2.5 homozygous and HLA-DQ2.2/2.5 heterozygous individuals was found, while HLA-DQ2.5/non-DQ2 heterozygous individuals had only a slightly increased risk of CD. If the threshold effect is valid, the amount of gluten needed to initiate the immunological response may be different in HLA-DQ2 homozygous and heterozygous individuals. On the other hand, a lack of *in vivo*/*ex vivo* evidence of gluten epitope diversity being greater in HLA DQ2 homozygotes with CD, and considering that information about children and gluten-specific T cells is limited to a single study, call for caution in the interpretation. Furthermore, a recent study supports greater T-cell epitope diversity in HLA DQ2/DQ8 + heterozygotes than in HLA DQ2 + individuals because of the efficient transdimer presentation of gluten peptides. However, HLA DQ2/DQ8 + individuals are at no greater risk of CD than HLA DQ2 + heterozygotes, suggesting epitope diversity is not clearly influencing susceptibility.[34]

Administration of probiotics and/or prebiotics

In other conditions characterised by a deranged immune response of the mucosal immune system, attention has been given to the possible role of manipulation of the gut microbiota. Probiotics and/or prebiotics have been suggested to influence immune development and the type of immune response. Therefore, it could be envisaged that probiotics/prebiotics may influence the type of immune reactivity to gluten in subjects with CD. The composition of the gut microbiota differs between individuals with CD and healthy individuals with respect to phylogenetic diversity and abundance of microbial taxa. For example, some of the most recent data, albeit obtained from a relatively small group of subjects, have shown that gut microbiota of infants at risk for CD exhibited reduced proportions of Bacteroidetes and an increased proportion of Firmicutes compared with those with a nonselected genetic background.[8] However, other studies have reported a higher abundance of Bacteroidetes.[9, 11] We were unable to identify intervention studies on supplementation of pre/probiotics and prevention of CD. Future studies need to establish the exact role of gut microbiota in the development of CD. If so, strategies to manipulate and reshape gut microbiota to a more healthy type may be of interest.

CONCLUSIONS

A summary of recommendations made for gluten introduction in the countries involved in the PREVENTCD project is presented in Table 4. With regard to the scientific authorities, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends that it is prudent to avoid both early (less than 4 months) and late (7 or more

Table 4 | Gluten introduction – current national and international recommendations

Society/country	Year	Recommendation
ESPGHAN Committee on Nutrition[35]	2008	It is prudent to avoid both early (<4 months) and late (≥ 7 months) introduction of gluten and to introduce gluten gradually while the infant is still being breastfed because this may reduce the risk of CD, type 1 diabetes mellitus, and wheat allergy.
Croatia[37]	2010	After a full 4 months, preferably while still being breastfed.
Germany[38]	2011	Introduction of gluten in small quantities preferentially while the infant is still being breast-fed, not earlier than at the beginning of the 5th and no later than at the beginning of the 7th month.
Israel[39]	2009	National guidelines are in accordance with ESPGHAN guidelines.
Netherland[40]	1999	After 6 months of age.
Poland[41]	2007	After a full 4 months and before the end of 6 months. Small amounts, preferably while still being breastfed.
Sweden[42]	2011	Introduction of gluten in small amounts preferably while the infant is still being breastfed, not earlier than age 4 month and no later than age 6 month.
US (American Academy of Pediatrics)[36]	2012	Complementary foods can be introduced between 4 and 6 months of age. Gluten-containing foods should be introduced while the infant is receiving only breast milk and not infant formula or other bovine milk products.

months) introduction of gluten and to introduce gluten while the infant is still being breastfed.[35] The Committee considers that such a strategy may reduce not only the risk of CD, but also the risks of type 1 diabetes mellitus and wheat allergy. The American Academy of Pediatrics (AAP) recommends that complementary foods can be introduced between 4 and 6 months of age; gluten-containing foods should be introduced while the infant is receiving only breast milk and not infant formula or other bovine milk products.[36] In the absence of clear evidence, it is reasonable to follow recommendations made by scientific organisations such as ESPGHAN while awaiting results of future studies (e.g. PREVENTCD project) that will shed light on the remaining uncertainties.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of included individual studies.

Table S2. Excluded studies and reasons for exclusion.

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