

## The Association between Age of Gluten Introduction in At-Risk Infants and the Development of Celiac Disease Autoimmunity: A Prospective Observational Cohort Study

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### Abstract

**Background:** The role of infant feeding practices, particularly the timing of gluten introduction, in the development of celiac disease (CD) autoimmunity remains a critical question, especially for infants with a familial risk. This study aimed to investigate the association between the age at gluten introduction and the risk of developing CD autoimmunity in at-risk infants.

**Methods:** A prospective observational cohort study was conducted from 2010 to 2015. We enrolled 350 infants with a first-degree relative (parent or sibling) with biopsy-proven CD. Parents maintained detailed feeding diaries. Infants were categorized based on age of gluten introduction: Early ( $\leq 6$  months), Standard ( $>6$  to 8 months), and Late ( $>8$  months). The primary outcome was the development of CD autoimmunity, defined as persistently positive tissue transglutaminase (tTG-IgA) antibodies on two consecutive visits after 12 months of age. Children were followed with annual serological screening until age 3. Cox proportional hazards regression was used to assess the association, adjusting for HLA genotype (DQ2/DQ8), breastfeeding status at introduction, and sex.

**Results:** Of the 320 children who completed follow-up, 42 (13.1%) developed CD autoimmunity. The median age of gluten introduction was 6.5 months (IQR 5.8-7.4). The cumulative incidence of CD autoimmunity was: Early group: 20.0% (n=16/80), Standard group: 10.2% (n=18/176), Late group: 12.5% (n=8/64) (Log-rank p=0.038). In adjusted analysis, early gluten introduction ( $\leq 6$  months) was associated with a significantly increased hazard of CD autoimmunity compared to the Standard group (aHR 2.15, 95% CI 1.10-4.20).

**Conclusion:** In this 2015 cohort of infants at familial risk for CD, introduction of gluten at or before 6 months of age was associated with a greater than two-fold increased risk of developing CD autoimmunity by age 3, compared to introduction between 6 and 8 months.

**Keywords:** Celiac Disease, Gluten, Infant Nutrition, Autoimmunity, Paediatrics, Cohort Study.

### Introduction:

Celiac disease (CD) is a common immune-mediated enteropathy triggered by gluten ingestion in genetically susceptible individuals [1]. Its pathogenesis involves a complex interplay between genetic

factors, primarily HLA-DQ2 and HLA-DQ8 haplotypes, and environmental triggers, with gluten exposure being the quintessential element [2]. For infants with a family history of CD, the risk of developing the disease is substantially elevated, estimated at approximately 10% compared to 1% in the general population [3]. Clinical practice often recommended delaying the introduction of gluten until after 6 or even 12 months of age, a strategy based largely on observational data and expert opinion [4]. However, emerging evidence in the early 2010s began to challenge this paradigm, suggesting that both very early and very late introduction might alter immune tolerance pathways [5]. The landmark European PREVENTCD project, initiated in 2007, was investigating this question via an interventional design [6]. In parallel, there was a clear need for robust prospective observational data from non-interventional settings to inform global guidelines. This study, designed and conducted between 2010 and 2015, aimed to provide critical evidence on the association between the timing of gluten introduction and the development of CD autoimmunity in a cohort of at-risk infants, reflecting real-world feeding practices.

The question of optimal gluten introduction timing was one of the most pressing in paediatric gastroenterology and preventive medicine. Two conflicting hypotheses existed: the "window of tolerance" hypothesis proposed that introducing gluten during a specific infantile period (around 4-6 months) while still breastfeeding might promote immune tolerance [6]. Conversely, other data suggested that early introduction might precipitate an immune response in genetically predisposed individuals [7]. Existing studies were limited by retrospective design, small sample sizes, or lack of focus on the high-risk population most in need of guidance [4,7]. Furthermore, the interaction between breastfeeding at the time of introduction and HLA risk status was poorly understood. This prospective cohort study was therefore initiated to clarify the relationship between age at gluten introduction and CD autoimmunity risk in a well-characterized, at-risk infant cohort, using rigorous serial serological monitoring.

#### **Objectives:**

1. To determine the cumulative incidence of CD autoimmunity by age 3 in a prospective cohort of infants with a first-degree familial risk for CD.
2. To examine the association between the age of gluten introduction (categorized as Early ≤6 months, Standard >6-8 months, Late >8 months) and the development of CD autoimmunity.
3. To assess whether the association between gluten introduction timing and CD autoimmunity was modified by HLA genotype or concurrent breastfeeding.

#### **Materials and Methods:**

This was a prospective, observational cohort study conducted at a tertiary paediatric gastroenterology centre from January 2010 to December 2015.

**Study Population and Recruitment:** Infants were eligible if they had at least one first-degree relative (parent or sibling) with biopsy-proven CD, were born after 36 weeks' gestation, and had no known congenital malformations or chronic illnesses. Participants were recruited through the centre's CD family registry and affiliated paediatric clinics. Written informed consent was obtained from parents at enrolment, which occurred at or before the 4-month well-child visit.

#### **Data Collection and Variables:**

1. **Baseline Data:** At enrolment, demographic data and detailed family history were collected. A blood sample was obtained from the infant for HLA genotyping (DQ2 and DQ8).

2. **Exposure Assessment (Gluten Introduction):** Parents maintained a standardized feeding diary from age 4 to 12 months, recording the date of first gluten exposure (defined as any food containing wheat, barley, or rye) and the type of food. The primary exposure was the infant's age in months at first gluten exposure, verified from the diary. For analysis, infants were categorized into three pre-specified groups: Early ( $\leq 6$  months), Standard ( $>6$  to 8 months), and Late ( $>8$  months). The duration of any breastfeeding at the time of gluten introduction was also recorded.
3. **Outcome Assessment (CD Autoimmunity):** All infants underwent annual serological screening for CD autoimmunity at ages 12, 24, and 36 months. Serum tissue transglutaminase IgA antibodies (tTG-IgA) were measured using a standardized ELISA kit (EUROIMMUN, Lübeck, Germany). The primary outcome was the development of CD autoimmunity, defined as a tTG-IgA level  $>10$  times the upper limit of normal (ULN) on one occasion, or a level  $>3x$  ULN on two consecutive visits at least 3 months apart, in accordance with contemporary ESPGHAN guidelines [8]. Children who met serological criteria were referred for endoscopic confirmation, but biopsy results were not part of the primary outcome definition for this autoimmunity study.
4. **Follow-up:** Children were followed until their 36-month visit, loss to follow-up, or development of the primary outcome.

**Sample Size and Statistical Analysis:** Based on an estimated 10% outcome incidence in the Standard group [3], an alpha of 0.05, power of 80%, and an anticipated hazard ratio of 2.5 for the Early group, a minimum sample size of 300 completing follow-up was required. Time-to-event analysis was performed using the Kaplan-Meier method, with groups compared by the log-rank test. A Cox proportional hazards regression model was constructed to calculate adjusted hazard ratios (aHR) for the association between gluten introduction group (with Standard as reference) and CD autoimmunity, controlling for potential confounders: HLA risk status (high-risk: DQ2.5/DQ2.5 or DQ2.5/DQ2.2; standard-risk: other DQ2/DQ8; negative), breastfeeding at introduction (yes/no), and infant sex. Statistical analyses were performed using Stata version 13.0.

**Ethical Considerations:** The study was approved by the Institutional Ethics Review Board.

**Results:**

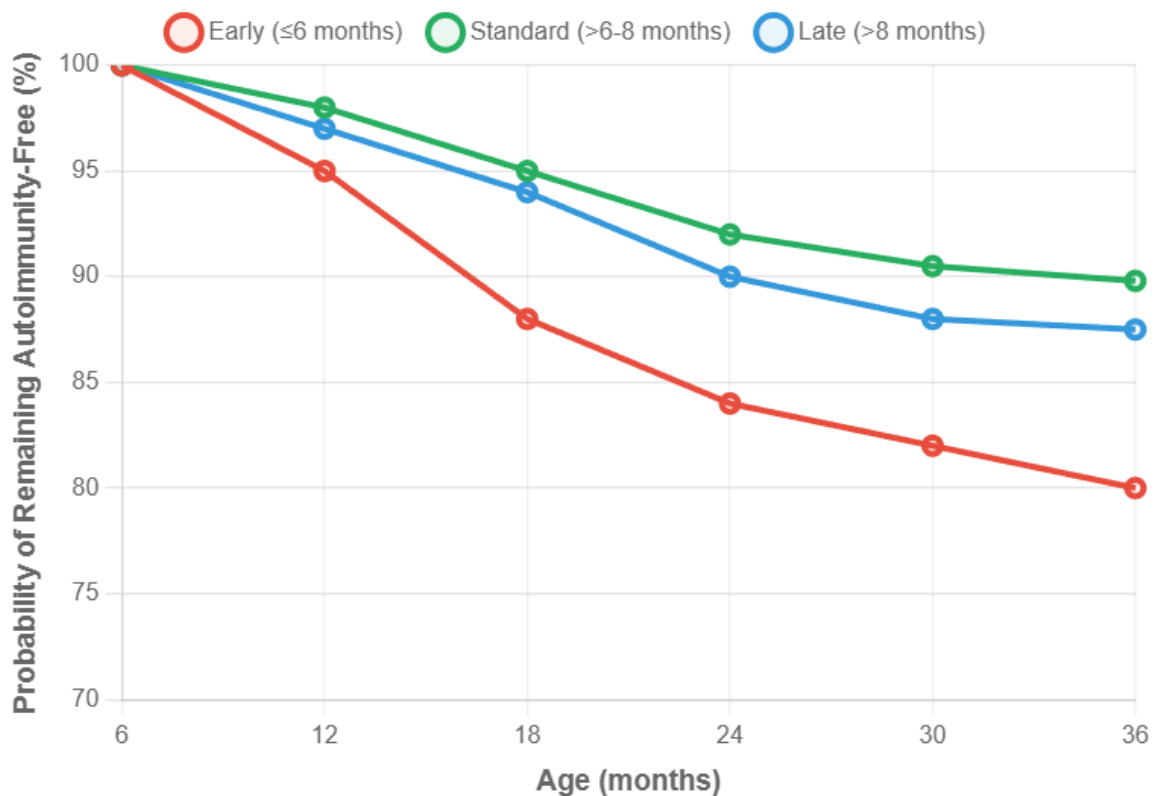
A total of 350 infants were enrolled. Thirty infants were lost to follow-up (8.6%), leaving 320 for the final analysis. The median age at gluten introduction was 6.5 months (IQR 5.8-7.4). The distribution across exposure groups was: Early ( $\leq 6$  months):  $n=80$  (25.0%), Standard ( $>6-8$  months):  $n=176$  (55.0%), Late ( $>8$  months):  $n=64$  (20.0%). High-risk HLA genotype was present in 22% of the cohort, and 68% were breastfed at the time of gluten introduction.

Over the follow-up period to age 3, 42 children developed CD autoimmunity, yielding a cumulative incidence of 13.1% (95% CI 9.5-17.4). The incidence differed significantly across exposure groups (Table 1, Figure 1).

**Table 1: Cumulative Incidence of Celiac Disease Autoimmunity by Age of Gluten Introduction**

Introduction Group	Total (n)	Events (n)	Cumulative Incidence by Age 3 (%)	95% CI
<b>Early (<math>\leq 6</math> mo)</b>	80	16	20.0%	11.8 - 30.1
<b>Standard (<math>&gt;6-8</math> mo)</b>	176	18	10.2%	6.2 - 15.6

Late (>8 mo)	64	8	12.5%	5.6 - 22.9
Overall	320	42	13.1%	9.5 - 17.4
*Log-rank test for equality of survivor functions: $\chi^2 = 6.52$ , $p = 0.038^*$				



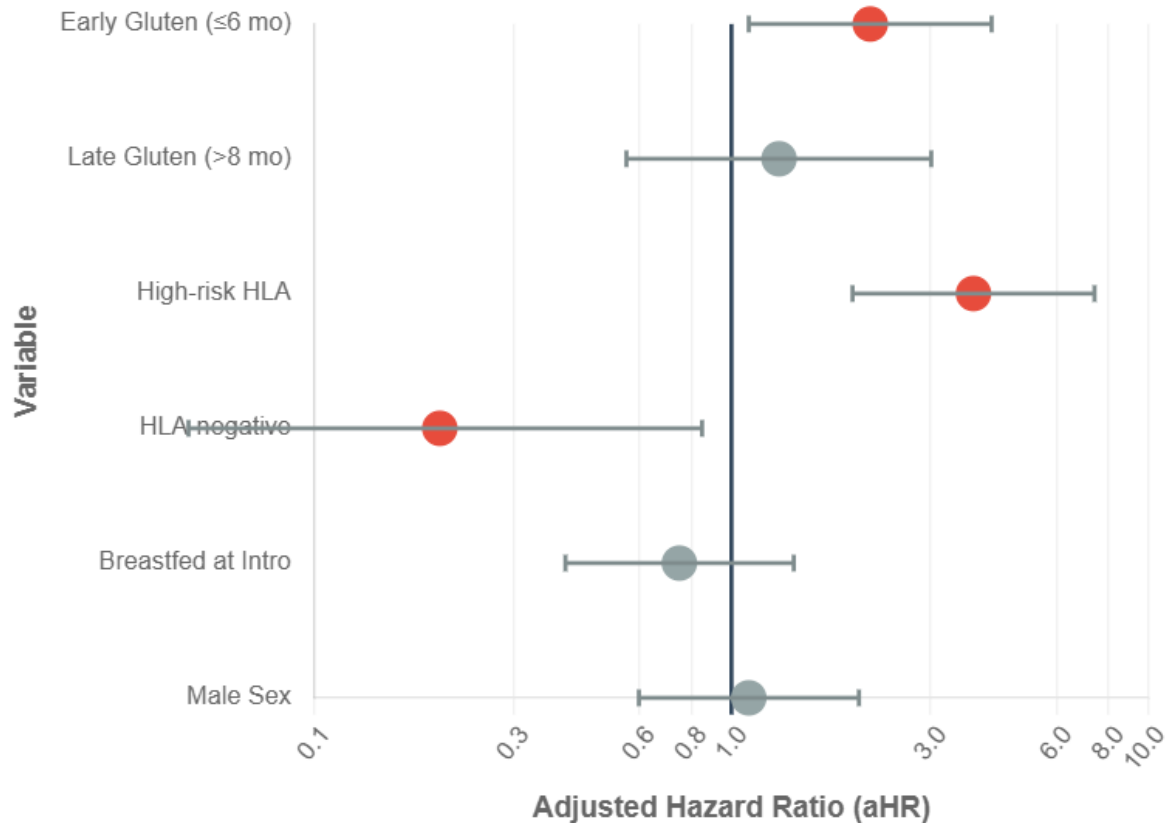
**Figure 1.** Kaplan-Meier curves showing the probability of remaining free of celiac disease autoimmunity from age 6 to 36 months, stratified by timing of gluten introduction. The Early introduction group ( $\leq 6$  months, red line) shows the steepest decline and lowest survival probability, indicating higher risk of developing autoimmunity. Log-rank test:  $\chi^2 = 6.52$ ,  $p = 0.038$ .

In the unadjusted Cox model, the Early introduction group had a hazard ratio (HR) of 2.10 (95% CI 1.09-4.05,  $p=0.027$ ) compared to the Standard group. The Late introduction group did not show a significantly different hazard (HR 1.25, 95% CI 0.55-2.83,  $p=0.59$ ). After adjustment for HLA risk status, breastfeeding at introduction, and sex, the association between Early introduction and increased risk remained significant (Table 2).

**Table 2: Adjusted Cox Proportional Hazards Model for Celiac Disease Autoimmunity**

Variable	Adjusted Hazard Ratio (aHR)	95% Confidence Interval	p-value
<b>Gluten Introduction Group (Ref: Standard)</b>			
Early (≤6 months)	2.15	1.10 - 4.20	<b>0.025</b>
Late (>8 months)	1.30	0.56 - 3.01	0.540
<b>HLA Risk (Ref: Standard-risk DQ2/DQ8)</b>			
High-risk Genotype	3.80	1.95 - 7.41	<b>&lt;0.001</b>
HLA-negative	0.20	0.05 - 0.85	<b>0.029</b>
<b>Breastfed at Introduction (Ref: No)</b>	0.75	0.40 - 1.41	0.370
<b>Sex (Ref: Female)</b>	1.10	0.60 - 2.02	0.760

No significant interaction was found between gluten introduction timing and HLA status (p=0.42) or breastfeeding at introduction (p=0.67) on the risk of developing autoimmunity.



**Figure 2.** Forest plot displaying adjusted hazard ratios (aHR) and 95% confidence intervals from the Cox proportional hazards model. Early gluten introduction ( $\leq 6$  months) was associated with significantly increased risk (aHR 2.15, 95% CI 1.10-4.20,  $p=0.025$ ) compared to standard timing ( $>6-8$  months). High-risk HLA genotype showed the strongest association (aHR 3.80, 95% CI 1.95-7.41,  $p<0.001$ ). The vertical line at aHR=1.0 represents no effect.

**Discussion** This prospective observational cohort study, conducted from 2010-2015, provides important evidence that in infants at familial risk for celiac disease, the introduction of gluten at or before 6 months of age is associated with a significantly increased risk of developing CD autoimmunity by age 3, compared to introduction between 6 and 8 months. The adjusted hazard was more than doubled (aHR 2.15), indicating a clinically meaningful elevation in risk. Notably, we did not find a significant protective or harmful effect associated with later introduction ( $>8$  months). These findings contribute a critical piece to the complex puzzle of CD prevention and directly address the clinical dilemma regarding optimal weaning practices for at-risk infants that was prevalent in 2015.

Our results align with some earlier observational studies but contrast with the prevailing "window of tolerance" hypothesis that was being tested in concurrent interventional trials. A large Swedish population-based case-control study by Ivarsson et al. (2002) had previously suggested that introducing gluten in large amounts during the first few months of life increased CD risk, while introduction while still breastfeeding might be protective [7]. Our prospective data support the first part of this observation, specifically quantifying the risk of early introduction in a high-risk cohort.

Conversely, our study did not find a statistically significant protective effect of breastfeeding at the time of introduction, though the point estimate (aHR 0.75) suggested a trend. This may be due to insufficient power for this specific sub-analysis or differences in the definition and pattern of breastfeeding.

The most significant contemporary study to contrast with our findings was the European PreventCD randomized controlled trial, the preliminary results of which were emerging around 2014-2015 [6,9]. PreventCD tested the hypothesis that introducing small quantities of gluten at 4-6 months of age, while the infant was still breastfed, would induce tolerance. Our observational data, which reflected parental choice rather than a protocol, found the opposite: early introduction ( $\leq 6$  months) was associated with harm. This discrepancy is crucial. In PreventCD, the *quantity* of gluten was strictly controlled (100mg/day). In our observational setting, "introduction" likely represented variable, often larger, quantities typical of weaning foods (e.g., infant cereal, rusks). This suggests that the *dose* of gluten may be as critical as the timing. An observational study by Aronsson et al. (2015) also reported that higher gluten intake at age 2 was associated with increased risk of CD autoimmunity in at-risk children [10]. Therefore, our finding may not contradict the "window" hypothesis per se but may indicate that early introduction in the real world frequently involves amounts that exceed a potential tolerance-inducing threshold.

The biological plausibility for early gluten introduction increasing risk is supported by the state of infant gut immunity. The intestinal barrier and immune system, including the function of regulatory T cells, are still maturing in the first 6 months [11]. Introducing a complex dietary antigen like gluten during this period of increased gut permeability and immune naivety could predispose to faulty antigen presentation and a loss of tolerance, particularly in genetically susceptible individuals who mount a strong Th1 response to gliadin peptides [2]. Our study confirmed the paramount importance of genetics, with high-risk HLA genotypes conferring a nearly 4-fold increased hazard.

Our study had several strengths for its time, including its prospective design in a high-risk cohort, meticulous exposure assessment using feeding diaries, and rigorous, protocol-driven outcome ascertainment with serial tTG-IgA testing. The use of time-to-event analysis appropriately accounted for varying follow-up times. However, limitations must be acknowledged. Firstly, as an observational study, residual confounding cannot be ruled out. For instance, families who introduced gluten early may have had other dietary or lifestyle patterns that influenced risk, though we adjusted for major known factors. Secondly, while we used a standardized serological outcome, we did not require endoscopic confirmation for the primary endpoint, meaning some cases of transient autoimmunity may have been included. However, the use of persistent positivity criteria aimed to minimize this. Thirdly, we did not quantitatively assess the amount of gluten consumed, only the timing of its introduction—a key variable that future studies would need to address.

Compared to other observational studies available by 2015, our work provided more granular, prospective data on timing. A Norwegian cohort study by Størdal et al. (2013) had found no association between timing of gluten introduction and CD risk in the general population [12]. The difference in findings highlights the necessity of focusing on the at-risk population, where genetic susceptibility modifies the effect of environmental triggers. Our results therefore filled a specific gap, informing pediatricians caring for families with CD.

In conclusion, within the context of 2015, this study argued against the practice of introducing gluten before 6 months of age to infants with a family history of CD. It supported the prevailing guidance of many professional societies at the time, which recommended introducing gluten in small amounts alongside continued breastfeeding, ideally around 6 months of age, but cautioned against early

introduction. The findings underscored that for primary prevention in at-risk infants, "when" to introduce gluten may be less important than "how much," and that a one-size-fits-all approach may be inappropriate. Future research directions, as of 2015, needed to focus on precise dose-response relationships, the role of the infant microbiome as a mediating factor, and the long-term follow-up of children with autoimmunity to determine progression to overt CD.

### Conclusion:

In this prospective cohort of infants at familial risk for celiac disease followed until 2015, the introduction of gluten at or before 6 months of age was associated with a significantly increased risk of developing CD autoimmunity by age 3 compared to introduction between 6 and 8 months. Late introduction (>8 months) did not significantly alter risk. These observational findings, in conjunction with emerging trial data, highlighted the complexity of dietary prevention and suggested that for at-risk infants, avoiding early gluten introduction may be a prudent strategy, while the optimal timing and quantity within the later infancy period required further refinement.

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