Expert Review of Clinical Immunology

ISSN: 1744-666X (Print) 1744-8409 (Online) Journal homepage: http://www.tandfonline.com/loi/ierm20

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To cite this article: Antonella Diamanti, Teresa Capriati, Carla Bizzarri, Francesca Ferretti, Monica Ancinelli, Francesca Romano, Alessia Perilli, Francesca Laureti & Mattia Locatelli (2015): Autoimmune diseases and celiac disease which came first: genotype or gluten?, Expert Review of Clinical Immunology, DOI: 10.1586/1744666X.2016.1095091

To link to this article: http://dx.doi.org/10.1586/1744666X.2016.1095091

Published online: 16 Oct 2015.

EXPERT REVIEWS

Autoimmune diseases and celiac disease which came first: genotype or gluten?

Expert Rev. Clin. Immunol. 12(1), 000–000 (2015)

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Celiac disease (CD) is associated with several autoimmune diseases (ADs) and, in particular, thyroid autoimmunity (TA) and Type 1 diabetes (T1D). TA and T1D are defined as 'associated conditions' to CD (conditions at increased prevalence in CD but not directly related to gluten ingestion). The diagnosis of CD may precede or follow that of TA/T1D. To date, the available evidence suggests that the common genetic background is the main factor determining the high prevalence of the association. Conversely, no conclusive findings clarify whether extrinsic gluten-related factors (age at the first introduction, concomitant breastfeeding, length of gluten exposure and gluten-free diet) may link CD to the ADs. The aim of this review is to evaluate whether genetic background alone could explain the association between CD and ADs or if gluten-related factors ought to be considered. The pathophysiological links clarifying how the gluten-related factors could predispose to ADs will also be discussed.

KEYWORDS: adolescent • autoimmune diseases • celiac disease • child • thyroid autoimmunity • type 1 diabetes

Celiac disease (CD) is a life-long condition of the gastrointestinal tract that affects the small intestine in genetically susceptible individuals [1]. Several autoimmune diseases (ADs) are associated with CD [2–4]. Overall, about 30% of all the patients with CD have one or more ADs [5–7], while in the general population, ADs have a prevalence ranging from 3 to 9.4% [8,9].

Type 1 diabetes (T1D) and thyroid autoimmunity (TA) are the most frequently CD-associated ADs [10,11]. Epidemiological data report that CD can affect 4–11% of T1D patients and about 1% of the general population [12]. Thus, the screening for CD in T1D children is currently scheduled at the onset, every year during the 4 years since the diagnosis and every other year in the following six [13]. Positive CD-related auto-antibodies require biopsy to confirm CD; histology could be avoided in selected and well-defined cases, according to the recent guidelines from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [1]. CD occurs in 7 to 26% of TA patients aged less than 18 years [14–27] and it should be regularly serologically screened during TA follow-up [1].

The nature of the link between CD and ADs has not been well defined. A key role of the common genetic background is suggested by the higher prevalence of ADs in CD subjects and in their relatives than in the general population [28–33]. CD and T1D/TA share HLA DR3-DQ2 and DR4-DQ8 haplotypes. DR3-DQ2/DR4-DQ8 are associated with the highest risk of developing T1D [30,31] and TA [33]. HLA DR4 and DR3 are strongly associated with T1D and 30–50% of T1D patients have been found heterozygous for DR3/DR4 [30,31]. Approximately 90% of T1D individuals have either DQ2 or DQ8; HLA-DQ2 is also expressed by more than 95% of CD subjects and HLA-DQ8 by the remaining [30]. Although also factors related to exposure to gluten have been advocated to explicate the relationship between CD and ADs [6,34–72], they (age at gluten introduction and breastfeeding) did not result as being associated with the risk of developling CD alone in a recent multicenter Italian study [73].

The aim of this review is to evaluate whether the risk to develop CD-associated ADs is related to the genetic background alone or it could also triggered by gluten-related factors. T1D and TA will be considered as a

 10.1586/1744666X.2016.1095091 ISSN 1744-666X

model of ADs due to their high co-occurrence in CD. The impact of gluten-related factors on the development of ADs will be evaluated by exploring: age at first gluten introduction; gluten introduction and breastfeeding; length of gluten exposure before CD diagnosis (age at diagnosis); occurrence and clinical course of ADs on a gluten-free diet (GFD). All the surveys including subjects <18 years with CD and T1D/TA and the key pathophysiological features clarifying the link between gluten and ADs will be discussed.

Why may gluten predispose to ADs?

Gluten may represent a potential trigger for ADs because: it can alter the intestinal barrier [74]; it seems to have similarity with self-antigens, which may begin the autoimmune process in many organs and tissues [60–62,75,76]; it is able to activate T-cell immunity [72,65,66] and trigger antibodies response acting against tissues other than the intestine; and it can determine changes in intestinal microbiota [41–49].

Loss of the barrier effect of the intestinal mucosa

Increased intestinal permeability is a biologic change that precedes ADs and CD onset [65,67–69]. The mechanism by which gluten could alter the intestinal barrier involves one of the two main components of the gluten that binds to an intestinal receptor (CXCR3), so determining the release of zonulin in large quantities [77]. Zonulin or prehaptoglobin-2 is the only physiological modulator of the intercellular tight junctions (TJ) permeability so far described and is involved in the trafficking of macromolecules and the balance between tolerance and immune response to oral antigens [77]. Zonulin increases intestinal permeability through a disassembly of the small intestine TJ [77] allowing not-self-antigens (including the indigestible fragments of gluten) to reach the underlying lamina propria [77]. In the lamina propria, zonulin elicits immune response and subsequent tissue damage in genetically susceptible individuals.

Disrupted mucosal barrier allows exposure of dietary antigens to submucosal immune cells leading to unfavorable immune activation and the development of ADs [78,79]. Morphological changes in gut epithelial cells and intraepithelial lymphocytes infiltration have been demonstrated in both patients with T1D and animal models [80–82]. Ultrastructural changes of enterocytes associated with functional alterations of intestinal barrier have been detected even in patients with Hashimoto's thyroiditis [50].

Molecular mimicry

Molecular mimicry between wheat and human proteins could induce and/or maintain mucosal damage in several organs and tissues outside the intestine [60–62]. However, almost all CD patients present a complete remission of both inflammation and auto-antibodies after withdrawal of cereal proteins from the diet. This evidence makes it difficult to understand how, outside the intestine, gluten may perpetuate inflammation and self-antigens toward organs or tissues target [83].

Anti-transglutaminase antibodies (tTG-Ab) may play a relevant role in molecular mimicry and the damage of β cells in the islet of pancreas [71,84,85]: the transglutaminase (TG) has been shown as a major component of the insulin-like growth factor-binding protein-3 kinase activity present on breast cancer cell membranes [84]; TG activity was found markedly increased in guanosine triphosphate depletion-induced apoptosis of insulin-secreting cells [85]; TG are involved in the release of insulin from pancreatic islet cells [71].

Furthermore, Naiyer *et al.* [86] showed that tTG-Ab by sera of untreated CD patients react with transglutaminase 2 in thyroid tissue, so providing evidence in favor of the molecular mimicry by tTG also in TA development.

T-cell-mediated immune mechanisms

In animal models, there is evidence of an immunological link between pancreas and gut. Diabetogenic T cells that have been activated in the gastrointestinal tract settle in the pancreatic islets. Luminal antigens presentation takes place in the pancreatic lymph nodes [65,66]. Gluten may impact on T1D development by influencing proportional changes in immune cell populations or by inducing an inflammatory profile of the cytokine/chemokine pattern [87].

Changes of intestinal microbiota

A series of studies indicate a critical role of intestinal commensal microbiota in autoimmune system development [88] and in ADs onset, such as inflammatory bowel diseases, T1D, rheumatoid arthritis and multiple sclerosis [79,89–91].

Studies from humans and animals have shown a relationship between changes in intestinal microbiota composition and T1D development [41,45–48]. In particular, there is evidence that the cross-talk between gut microbiota and innate immune system may be involved in the destruction of the islets. Although the causal link between gut microbiota and T1D is evident, it is unclear which bacteria are involved and how they can promote the development of ADs.

In humans, the role of the gut microbiota in Hashimoto's thyroiditis is not well documented [92]; while in animal studies, it is better known [93–100].

Table 1 reports all the pathophysiological changes triggered by the gluten and potentially involved in ADs onset.

Could age at first gluten introduction impact on ADs development?

Although gluten introduction has been recommended while the infants is still breastfeeding between 4 and 6 months of age [101–108], two systematic reviews in 2012 [109] and 2015 [110] concluded that the infant feeding practices (breastfeeding, time of gluten introduction) have no effect on the risk of developing CD during childhood. In effect, a recent prospective birth cohort study [111] (The Environmental Determinants of Diabetes in the Young, TEDDY study) concludes that age at first introduction of gluten is not an independent risk factor for developing CD by 5 years of age. In

Table 1. Main pathophysiological features by which the gluten may trigger T1D & TA.

Pathophysiological features	Evidences in T1D and TA	Ref. (N†)
Loss of barrier effect of intestinal mucosa	Evidences of mucosal inflammation in small intestinal biopsies from T1D and TA	[64]
	Altered intestinal permeability in T1D	[67–69]
	Alterations of TJ in T1D	[69]
	Elevated serum zonulin levels in 50% of T1D patients	[70]
Molecular mimicry	Homologies between wheat and human proteins	[60–62]
	Genes with gliadin homology may be involved in beginning both CD and T1D	[63]
	TG Has Been found to be Involved in releasing insulin from pancreatic islet cells	[71]
	tTG-Ab react towards thyroid tissue, thereby contribute to the development of thyroid disease	[86]
T-cell mediated immune mechanisms	Diabetic patients with HLA-DR at risk genotype show enhanced T-cell reactivity to wheat derived polypeptides, with production of IFN-gamma and IL-17	[72]
	Dendritic cells that have tied gliadin can move from small intestine and reach the mesenteric lymph nodes and pancreatic islet. This event cause the migration of activated T lymphocytes to target organ (small intestine and/or pancreas)	[65,66]
Alterations of intestinal microbiota	Intestinal commensal microbiota have a critical role in autoimmune system development	[88]
	It is show a relationship between changes in the intestinal microbiota composition and T1D development	[30,34–47]
	There are few evidence on the role of indigenous microorganism in the pathogenesis of TA	[92–100]

†: Number.
CD: Celiac disease; HLA-DR: Human leucocyte antigen DR; IFN-gamma: Interferon gamma; IL-17: Interleukin 17; T1D: Type 1 diabetes; TA: Thyroid autoimmunity; TG: Transglutaminaset; tTG-Ab: Anti transglutaminase antibodies; TJ: Thight junctions.

particular, gluten introduction before 17 or after 26 weeks was not associated with increased risk for CD compared with introduction between 17 and 26 weeks. Age at gluten introduction does not represent a risk factor nor a protective factor for the onset of CD, also in a multicenter, randomized, double-blind, placebo-controlled dietary interventional study carried out by Vriezinga *et al.* [112]. In this interventional study, early introduction (at 16 weeks of age) of small quantities of gluten and breastfeeding did not reduce the risk of CD at 3 years of age in genetically predisposed children from high-risk family. Lionetti *et al.* [73] did not find differences in CD prevalence between children who begun gluten at 6 and 12 months.

However, late introduction of gluten in at-risk infants may delay CD onset with potential benefits related to the state of health during a crucial period of child development, as also suggested by the study from Sellitto *et al.* [113]. This study assessed the impact of early and late gluten introduction on the gut microbiota of infants up to 24 months. They found that the later exposure to gluten (12 months) resulted in prolonging gluten tolerance and delaying the onset of CD autoimmunity. These effects may be related to the overall lack of Bacteroides and the high abundance of Firmicutes from microbiota of infants genetically predisposed to CD.

Norris *et al.* [114] analyzed the probability to develop T1D in 1183 children genetically at risk who started gluten supplementation before 3 months or after 7 months of age. They showed a higher hazard ratio (HR) for developing β-cell auto-antibodies in these subjects, thus indicating that the first introduction of gluten during the 'window period' between 3 and 7 months could prevent the future development of T1D.

These findings were replayed by BABYDIAB study showing that early introduction of gluten resulted in increased appearance of islet-related auto-antibodies, but not of CD serological markers [115]. The aim of the study was to analyze the development of islet-related auto-antibodies against insulin (IAA), glutamic acid decarboxylase (GADA), or insulin-associated protein 2 (IA2A) and its correlation with the diet. Children receiving food supplementation with gluten-containing foods before 3 months presented a significantly higher risk of β-cell autoimmunity in comparison with children receiving exclusive breast milk after the first 3 months of life. This finding could be biased by the fact that four out of the 17 children who had received gluten before 3 months and who developed β-cell auto-antibodies, all carried a high-risk genotype.

The BABYDIET trial [116] analyzed whether the delay of the complementary gluten food may prevent the development of β-cell autoimmunity in newborns at high genetic risk of TID. All eligible subjects were younger than 2 months, exclusively fed milk formula and at genetic risk for T1D. One hundred and fifty children were randomly assigned to a first gluten exposure at 6 months (control group) or 12 months of age. Eleven children in the control group and 13 in the late-exposure group developed islet-related auto-antibodies. Six children developed auto-antibodies before their first reported gluten exposure. HR for developing beta-cell auto-antibodies was 1.3 (95% CI: 0.6–3.0) for the late-exposure group. Seven children developed diabetes (three in the control group and four in the late-exposure group) indicating that delaying gluten exposure until the age of 12 months does not reduce the risk of β-cell autoimmunity in genetically susceptible children. The DAISY group [117] analyzed the association

Table 2. Dietetic habit in the first year of life and development of AD or overt T1D.

Author (year)	Sample size (N†)	Follow up length (years)	Is the duration of BF protective?	Main outcome measures	Is the first exposure to gluten on BF protective?	Is the first exposure >3 and <7 m protective?	Is the exposure >7 m at risk?	Is the first exposure >12 m protective versus 6 m?	Ref. (N†)
Norris *et al.* (2003)	1183	0.9-9	Not	AD	Yes	Yes	Yes	Not >7 m risk	[114]
BABYDIAB (2006)	1610	8	Not	AD	Not	Yes	NE	NE	[115]
BABYDIET (2011)	150	>3	NE	AD T1D	Yes	Yes	Not	Not	[116]
DAISY (2013)	1835	>2	Not	T1D	Yes	Yes	Not	Not	[117]
MIDIA (2014)	726	7,7	Not for AI Yes for T1D	AD T1D	Not	Not	Not	Not	[120]

†: Number.
AD: Autoimmunity evaluated by serological testing of insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GAD), anti-insulin antigen 2 (IA2) and anti transglutaminase autoantibodies (tTG-Ab); BF: Breastfeeding; m: Months; NE: Not evaluated; T1D: Type 1 diabetes.

between acute illnesses during infancy and later development of β-cell autoimmunity. Complete illness reports through the first 9 months of age were collected for 1729 children: 1174 without genetic risk of T1D and 555 with a first-degree relative affected by T1D. Persistent β-cell autoimmunity was defined as positive IAA, GADA, and IA2A at least in two consecutive evaluations. During the study, 124 children developed persistent β-cell antibodies, but they had similar prevalence of acute illnesses (gastro-intestinal disorders, respiratory diseases, febrile episodes, or upper respiratory symptoms) to children who did not develop auto-antibodies. Exposure to wheat or barley early (0–3 months) or late (>7 months) increased the risk of developing β-cell autoimmunity, compared with exposure at 4–6 months of age. Each acute gastro-intestinal episode determined increased risk of 37% of antibodies development among children exposed to wheat or barley before 4 months of age and of 12% among children not exposed until 7 months of age. There were no associations between gastro-intestinal illnesses and persistent antibodies if wheat or barley were introduced between 4 and 6 months of age. The authors argued that digestive infections may increase the risk of β-cell autoimmunity in the presence of a co-existing inflammation induced by diet. However, in this study, the genetic risk for T1D was the variable independently associated with the highest risk of developing β-cell autoimmunity.

Could breastfeeding be protective toward ADs development?

The BABYDIAB STUDY [116] highlighted that exclusive breastfeeding length did not significantly affect the risk of developing β-cell auto-antibodies. However, children weaned with gluten-containing foods before 3 months had significantly higher risk of developing autoimmunity than children on breastfeeding after 3 months of life.

The DAISY group [117] reported that the first exposition to gluten on breastfeeding, but not full breastfeeding or any breastfeeding length, decreases the risk of developing islet-related antibodies, as also shown in several successive prospective studies [116,118–120]. Conversely, a Swedish survey suggested that short breastfeeding length and development of autoimmunity could be associated [117]. Data from Trial to Reduce IDDM in the Genetically at Risk (TRIGR), a randomized controlled trial including genetically susceptible children, not directly addressed to evaluate the effects of breastfeeding, found that infants fed with highly hydrolyzed formulas are not at decreased risk of developing autoimmunity than infants receiving cow's milk formulas [121].

A protective role of the timing of introduction of several food antigens, most likely between 4 and 6 months of age, has been suggested [37,86–89]. The DAISY study reported that both early and late first exposure to any solid food predicted development of T1D whether the introduction of cereals from 4 to 6 months could be protective [114,117]. The recent MIDIA study has not found any association between early or late introduction of any solid food and development of islet autoimmunity or T1D [120]. Furthermore, this survey found that breastfeeding length ≥12 months predicted a lower risk of progression from islet autoimmunity to T1D in genetically susceptible children.

Table 2 summarizes the main findings about dietetic habit in the first year of life and AD development.

Could age at CD diagnosis impact on the ADs occurrence?

Some surveys found that T1D and other ADs are less common in young newly diagnosed CD children rather than in older

children, suggesting that the beginning GFD early may prevent the onset of further ADs [5,6,13,18,122–125]. More recent surveys failed to confirm these results [15,17,20,21,126–128].

Could GFD prevent ADs development?

GFD & secondary prevention of T1D in subjects with positive β-cell antibodies

To verify the effect of GFD on secondary prevention of T1D, 17 first-degree relatives of T1D patients, with at least two positive β-cell auto-antibodies, underwent a 6-month period of GFD. The effects of the treatment were measured as autoantibody titer and acute insulin response to intravenous glucose tolerance test. During GFD regimen, two subjects dropped out due to poor compliance and three developed T1D. During the following 6 months, on a gluten-containing regimen, one subject developed T1D and all showed declined insulin secretion. Furthermore, one subject developed T1D at the study ending. Interestingly, in this study, insulin sensitivity slightly improved on GFD regimen and decreased on a gluten-containing diet. It is possible that the different carbohydrates composition of the diet may have influenced insulin response to glucose [124].

Fuechtenbusch *et al.* [125] enrolled seven first-degree relatives of patients with T1D, aged 1.2–5.9 years with elevated levels of islet-related auto-antibodies. They received a GFD for 12 months, followed by a normal gluten-containing diet for an additional 12 months. IAA, GADA, IA2A, and tTG-Ab were measured at baseline and every 3 months. During the follow-up, only one subject showed significant decreases of antibody levels in the GFD period. Three subjects developed T1D during the gluten re-exposure period. Changes in antibody levels observed in this small cohort at the end of the GFD were not significantly different from the fluctuations found in matched historical control subjects, suggesting that the removal of dietary gluten does not modulate the serological markers in the pre-T1D phase.

Conversely, Ventura *et al.* [14] tested 90 CD patients for serum levels of anti-endomysium antibodies (EMA), ICA, GADA, IAA at CD diagnosis and after 6, 12, and 24 months of GFD. At the time of CD diagnosis, all subjects were found positive to EMA and 11 to at least one islet-related antibody. By the end of the second year of GFD, all patients resulted negative for both EMA and islet-related antibodies, thus demonstrating that GFD might influence the appearance of serological markers of T1D.

In line with this latter study, Toscano *et al.* [15] found that gluten challenge determined a significant development of GADA, ICA, and IAA in CD patients when compared with CD patients on a GFD [15]. In both these studies, nevertheless, islet-related autoantibodies did not show any association with the progression towards T1D.

GFD & primary prevention of TA in diagnosed CD

Oderda *et al.* [18] observed that TA, found in 14.6% of their untreated CD patients series, disappeared in one-third after beginning of GFD; similarly Ventura *et al*[14]. reported a prevalence of TA in 14.4% at CD diagnosis that decreased at 2.2% over a period of 24 months on a GFD.

Likewise, Toscano *et al.* [15] found the number and titer of auto-antibodies significantly higher in untreated than in treated patients.

Subsequent surveys did not confirm these results. Ansaldi *et al.* [16], in a multicenter Italian survey, found in 256 CD patients a prevalence of TA (26.2%) significantly higher than in 230 health controls (10%), but it was similar in treated and untreated CD patients. We prospectively followed 558 CD patients on a GFD, with median disease length of 2.8 years. After 2 years of follow-up, the prevalence of TA slightly increased in spite of the compliance with GFD (from 10% to 12%) [20].

Cassio *et al.* [17] retrospectively evaluated a cohort including 135 consecutive patients diagnosed with CD since June 1990 to December 2004. At CD diagnosis, 12% of them showed TA but this figure increased to 23% at the final assessment on a strict GFD (23%).

In Tables 3 and 4, we summarize the details from each study regarding age at CD diagnosis and GFD on the occurrence of T1D and TA.

The role of age and GFD on the development of T1D and TA studies is summarized in Tables 3 and 4.

Could GFD have an impact on the clinical course of Ads?

Two surveys evaluated the impact of the GFD on the clinical trend of patients with previously diagnosed TA with thyroid failure and need for replacement therapy [23,24]. Valentino *et al.* [23] found, in their series of TA patients, that clinical conditions improved according to histological healing of jejunal mucosa and GFD beginning that resulted in a progressive decrease of required replacement therapy and of thyroid auto-antibodies titer.

Conversely, Larizza *et al.* [24] have studied the prevalence of CD in children and adolescents affected by TA. In this survey, no significant changes in auto-antibodies titer or amount of replacement therapy according to the beginning of the GFD. However, the only patient who suspended L-thyroxine was affected by CD.

A recent survey from Tsouka *et al.* [129] compared two groups of pediatric CD patients: one included patients with both CD and T1D and the other patients with CD alone. Subjects with comorbidity had lower weight, height and BMI; they also had more anemia and thyroid failure compared with the group of CD alone. The authors therefore hypothesized that patients suffering from CD and T1D could represent a distinct phenotype that could require the assessment of thyroid function every year.

Interestingly, Buschard *et al.* [130] described an anecdotal case of a child 5-year and 10-month-old who was diagnosed with T1D without CD. He started on a GFD 2–3 weeks after T1D diagnosis and did not need insulin treatment. They reported

Table 3. Gluten exposure & development of T1D: summary of the main evidences.

Author (year)	Sample size (N†)	Duration of follow-up (years)	Is the lower age at CD diagnosis protective?	Is the GFD protective towards T1D appearance?	Ref. (N†)
Ventura *et al.* (2000)	90	2	NE	Yes	[14]
Toscano *et al.* (2000)	44	1.6 ± 0.4	Not	Yes	[15]
Valerio *et al.* (2002)	383	7.6 ± 4.2	Not	NE	[126]
Norris *et al.* (2003)	1183	0.8–9	NE	Yes	[114]
Kaspers *et al.* (2004)	19796	NE	Not	NE	[127]
Cerutti *et al.* (2004)	4322	NE	Not	NE	[128]
Fuechtenbusch *et al.* (2004)	7	5	NE	Not	[125]
Hummel *et al.* (2011)	150	3	NE	Not	[116]

†: Number.
CD: Celiac disease; GFD: Gluten free diet; T1D: Type 1 diabetes; NE: Not evaluated.

that at GFD beginning, the glycated hemoglobin was 7.8% and was maintained at 5.8–6.0% without insulin therapy. Fasting blood glucose was maintained at 4.0–5.0 mmol/l. At 16 months after diagnosis, the fasting blood glucose was 4.1 mmol/l and after 20 months, the child was still without daily insulin therapy. Based on this experience, the authors suggested the need for further trials to confirm the efficacy of GFD in delaying insulin treatments and prolonging T1D remission [130,131].

Expert commentary

The prevalence of ADs in CD patients is much higher than in the general population; this leads an endeavor to detect the nature of the link as well as to try to optimize clinical work-up, especially the screening strategy. The association between ADs and CD is generally attributed to the genetic background, but it is assumed that gluten-related factors may also play a role in this co-occurrence. However, overall clinical evidence seems to support the common genetic background as the main underlying factor contributing to the co-occurrence of CD in two models of ADs represented by T1D and TA.

The screening strategy should take into account the different nature and clinical significance of the serological markers. In CD subjects, the finding of auto-antibodies correlates with intestinal damage induced by gluten [1,2], whose removal leads to the recovery of intestinal lesions. Conversely, in T1D and TA, the appearance of auto-antibodies may not immediately result in the occurrence of symptoms of irreversible organ damage; this process may take up to several years to fully manifest itself. Therefore, whether the systematic screening for CD in T1D and TA is clinically useful, in CD patients, the screening for the potentially associated endocrine ADs is not clearly indicated. In many cases, there is indeed an extended latency between the appearance of autoantibodies and the development of impaired organ function; therefore, the screening for endocrine ADs in CD patients does not seem

Table 4. Gluten exposure & development of TA: summary of the main evidences.

Author (year)	Simple size (N†)	Duration of follow-up (years)	Is the lower age at CD diagnosis protective?	Is GFD protective toward TA* appearance?	Ref. (N†)
Ventura *et al.* (2000)	909	>0.5	Yes	NE	[14]
Toscano *et al.* (2000)	44	1.6 ± 0.4	Not	Yes	[15]
Oderda *et al.* (2002)	66	1–5	Yes	Yes	[18]
Ansaldi *et al.* (2003)	343	0.25–16	NE	Not	[16]
Meloni *et al.* (2009)	324	8	Yes	Not	[19]
Cassio *et al.* (2010)	135	8.9 ± 4.0	Not	Not	[17]
Diamanti *et al.* (2011)	545	2	Not	Not	[20]
Van der Pals *et al.* (2014)	335	9	Not	Not	[21]

†: Number.
CD: Celiac disease; GFD: Gluten free diet; TA: Thyroid autoimmunity; NE: Not evaluated.

to be clinically relevant or cost-effective. Nevertheless, TA, which is rather frequent in the general population and in CD patients, could occasionally be evaluated during the follow-up, especially if some clinical symptoms could suggest thyroid impairment.

Five-year view

Several authors before 2000 hypothesized that autoimmunity could be 'gluten-dependent' and that gluten could be the triggering factor explaining the co-occurrence of CD and ADs. Reaffirming this statement, early GFD resulted in inducing, in a short-term follow-up, the disappearance of islet- and thyroid-related autoantibodies [14,18,114]. Assuming that the organ-specific autoantibodies could play a predictive pathogenic role in developing ADs, the authors concluded that GFD might be able to prevent the development of other ADs [14] This assumption is nevertheless debatable in the light of later findings. In fact, GFD has not been proven to prevent the development of T1D in infants with high T1D risk [125] nor in first-degree relatives of patients with T1D presenting elevated β-cell autoantibodies levels [125]. Furthermore, the co-occurrence of T1D and CD has been observed in young subjects, thus excluding gluten exposure length before CD diagnosis could have an impact on the development of ADs [128]. TA also occurs in spite of maintaining a correct GFD [16–21] and does not seem not to be influenced by gluten exposure length [17,19,20]. Therefore, in line with the advanced knowledge in this field, when ADs and CD co-occur, genotype seems to come before gluten.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- About 30% of all the patients with CD have one or more ADs, while in the general population, ADs have a prevalence ranging from 3 to 9.4%. The nature of the link between CD and ADs has not been completely identified yet. A higher prevalence of ADs in relatives of CD subjects suggests genetic background as the main factor.
- Gluten is involved in changes of gut permeability and intestinal microbiota, in molecular mimicry between wheat and human proteins and in activation of T-cell mediated. All these mechanisms make it a potential trigger for ADs.
- T1D and TA can be considered as a model of ADs due to their high co-occurrence in CD.
- Weak evidence supports the assumption that the first introduction of gluten between 4 and 6 months could be advantageous to reduce the risk of ADs development.
- Breastfeeding length does not prevent the risk of developing ADs, while the first gluten introduction during breastfeeding may be considered a protective measure.
- The length of gluten exposure before CD diagnosis in several surveys does not impact the future development of T1D and TA.
- The recent clinical advances highlight that GFD does not prevent the occurrence of ADs.
- Anecdotal cases showed that GFD may impact the outcome of ADs clinical course, but it needs to be confirmed by future clinical trials.
- The compliance with GFD may probably have no impact on the risk of developing ADs, as we can argue based on previous findings, although this aspect has not been specifically addressed in this review.

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