

EXPERT
REVIEWS

Celiac disease and endocrine autoimmune disorders in children: an update

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Celiac disease (CD) is a life-long inflammatory condition of the gut that occurs in genetically susceptible individuals. Several autoimmune diseases (AI) are associated with CD. To date, no conclusive evidence is available that proves if the relationship between CD and AI is mediated by gluten exposure, or if CD and AI could co-occur due to other causes, in particular the loss of the intestinal barrier function and the common genetic background. Furthermore, it is not clear yet if CD needs a regular screening program for AI. This review will cover the key studies on both the pathogenetic and clinical evidence explaining this association. We will review the reports including patients aged <18 years with CD and endocrine AI.

KEYWORDS: Addison's disease • 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 (AI) are associated with CD [2,3]. Overall, about 30% of the patients with CD have one or more AI [4–6], whereas in the general population, AI has a prevalence ranging from 3 to 11.6% [6,7].

To date, to the best of our knowledge, there is no conclusive evidence that proves the relationship between gluten exposure and development of AI in CD patients. In particular, two major points, regarding the gluten exposure, have been discussed in literature in the last years: the impact on AI of the total gluten exposure length before the diagnosis of CD, considered coincident with the age at CD diagnosis [5,6] and the potential protective effect on AI of gluten withdrawal in treated CD subjects [6–9].

In a multicenter Italian study, Ventura *et al.* highlighted that the age at diagnosis was the only significant predictor variable of developing AI. They also concluded that the prevalence of AI in CD is related to the duration of gluten exposure [5]. Sategna-Guidetti *et al.* showed that the increased age at CD diagnosis was related to the prevalence of AI while 'actual gluten exposure,' that takes into account compliance to the diet, length of follow-up and age at diagnosis of AI, were not

predictive for the risk of their development [6]. Di Mario *et al.* found that 27% of subjects with undiagnosed CD and 20% of the treated CD patients were positive to Type 1 diabetes (T1D) autoantibodies [10]. Viljamaa *et al.* reported that the age at the end of follow-up, age at CD diagnosis and female gender increased the risk of AI, whereas the 'actual gluten exposure time' reduced the risk [8]. In a large series of adult and pediatric French CD patients, Cosnes *et al.* reported that the incidence of AI was lower in the compliant gluten-free diet (GFD) group than in the non-compliant GFD one [9].

In this review, the authors will discuss the key studies pertaining the pathogenetic and clinical evidences on the association between CD and endocrine AI, in subjects aged <18 years. The main purpose of this review will be to investigate the impact of gluten exposure on the development and/or natural history of the endocrine AI. The secondary purpose will be to clarify the screening strategy in the clinical work-up of the association between CD and AI.

CD & endocrine AI: pathogenetic evidences

The coexistence of CD and endocrine AI involves a complex interplay between genetic

aspects, environment and immunologic factors, as well as intestinal barrier function.

Genetic aspects

The genetic basis of CD is traditionally split into variants within the Human Leukocyte Antigen (HLA) region and non-HLA variants. HLA heterodimer DQ2 is present in 95% of CD patients, whereas 5–10% carries the DQ8 heterodimer. HLA-DQ2 and HLA-DQ8 were considered the strongest genetic risk factors for CD; however, genome-wide association studies continue to link CD with non-HLA gene variants that control immune response [11], particularly B- and T-cell function [12].

Interestingly, many of the susceptibility loci for CD are shared with those for AI with a substantial genetic overlap [11,13]. The HLA DR3-DQ2 and DR4-DQ8 haplotypes are indeed common in many AI. The genotype DR3-DQ2/DR4-DQ8 is associated with the highest risk of developing Type 1 diabetes [14,15] but also with thyroid autoimmunity (TA) [16] and Addison's disease (AD) [17,18]. These results underline the important common genetic background of these conditions and suggest that there may be a shared genetic contribution. The common background is modified by a further subset of disease-specific genes and environmental insults leading to either T1D or CD or other AI.

Environmental factors

Gluten is the main environmental factor responsible for CD signs and symptoms; however, gluten exposure does not fully explain the manifestation of CD and AI.

Early feeding practices (e.g., breast milk vs formula, duration of breastfeeding, pattern/timing of gluten introduction in infant feeding), infections and alterations in the intestinal microbiota composition might, indeed, play a role in CD and AI development.

Gluten & pattern of infant nutrition

Gluten represents the main environmental factor that triggers CD. The high proline content makes gluten proteins resistant to complete proteolytic digestion, leading to the accumulation of relatively large peptide fragments with a high proline and glutamine content in the small intestine triggering abnormal immune response in susceptible individuals.

The exposure of an immature immune system to gliadin, a gluten protein component, in susceptible individuals seem to be a prominent cofactor in modifying the immunological response earlier in life, thus predisposing such individuals not only to overt CD, but also to AI [5,9].

The pattern of infant nutrition may play a critical role on the development of CD and other AI. A longitudinal follow-up study of a cohort of children with dietary gluten exposure before 3 months of age highlighted a significantly increased risk of developing CD-associated autoantibodies compared with the exposure at 4–6 months of age or later [19]. Furthermore, breastfeeding is thought to delay or reduce the risk of

developing CD [20,21]. This protective effect seems to be due to the different pattern of intestinal microbiota in breastfed infants (see below).

In both European and US infant populations, it is not recommended to introduce the complementary feeding containing gluten too early (<4 months) or too late (≥ 7 months) and to start gluten while the infant is still breastfed [22–25].

Intestinal microbiota

The composition of the intestinal microbiota has also been recently related to the development of CD. The first associations were established in CD patients, untreated and treated by GFD [26–28].

The microbiota pattern of CD patients seems to be characterized by increased numbers of *Bacteroides spp.* and a reduced presence of *Bifidobacterium spp.* and *B. longum*, which were not completely normalized by GFD [26–28]. *Escherichia coli* and *Staphylococcus* numbers also resulted higher in feces and biopsies of untreated CD patients than in those of controls, but this difference recovered after gluten withdrawal [27]. The milk-feeding patterns and HLA-DQ genotype could have a possible effect on microbiota early in life and, consequently, on later development of CD [29,30]. Some studies have reported a protective effect of breastfeeding on CD onset, probably partly related to the ability of human milk to modulate the intestinal microbiota differences related to genotype HLA-DQ [21,31].

Human studies have shown a relationship between the changes in the composition of the intestinal microbiota and T1D development [32]. This hypothesis seems to be confirmed by the data taken from studies on animal models as biobreeding [33], LEW1.WR1 rat models of virus-induced T1D [34] and the nonobese diabetic mouse [35–37]. In particular, there are evidences that the cross-talk between the gut microbiota and the innate immune system may be involved in the destruction of the islet. Although the causal link between gut microbiota and T1D is evident, the identity of the bacteria involved and the mechanism by which they promote the disease remains unclear.

There are only a few studies that have evaluated the link between the gut and Hashimoto's thyroiditis [38].

Literature reports several evidences that Hashimoto's thyroiditis also involves ultrastructural changes of enterocytes associated with functional alterations of the intestinal barrier similar to those observed in nonceliac patients with T1D. Although these data need further confirmation, they suggest that the endocrine autoimmune diseases have similar autoimmune pathogenetic mechanisms of cell damage and impaired intestinal morphology and function [39].

Infectious diseases

It is known that infectious agents are potential AI triggers. It has also been suggested that in patients with CD, exposure to viruses or bacteria might contribute to a reduction of tolerance to gluten, being the cause of tissue damage and inflammation [40]. Infections can also influence the host immune tolerance through three main mechanisms: polyclonal activation of

lymphocytes, increased immunogenicity of the organ of self-antigens secondary to infection and inflammation mediated by antigen molecular mimicry (autoimmune response related to cross-reactivity between epitopes of the host man and immunologically similar epitopes of the infectious microorganism) [41].

There are several studies linking the onset of CD (in susceptible patients) with different infectious agents and several hypotheses have been made about the possible pathogenic mechanisms of these associations [42].

The adenovirus Type 12 was one of the first infectious agents associated with CD [43], although further studies have not confirmed this association. Hepatitis C virus (HCV) has an epidemiologic association with the onset of CD: in fact, CD prevalence in patients with chronic liver disease is higher (15-times) than in the general population [44]. CD is also present in 5% of patients with autoimmune liver disease [45]. Several other studies have not shown an increased prevalence of CD in patients with HCV infection [46], thus concluding that the association between CD and HCV can also be random [47].

On the basis of these studies, it has been suggested that gastrointestinal infections may trigger or facilitate the onset of CD by amplifying the immune response to gliadin or increasing intestinal permeability [48].

Immunological aspects

The pathogenesis of CD, as seen in AI, includes both adaptive and innate immune responses [49]. When a subject genetically predisposed (substantially carrier of haplotypes HLA DQ2 or DQ8) introduces gluten in the diet, gliadin is recognized by T lymphocytes through the classic process of antigen presentation [50]. HLA-DQ2 and HLA-DQ8 bind peptides with negatively charged amino acids at anchor residues, but gluten peptides are virtually devoid of negative charges. In the gut tissue, transglutaminase interacts with gliadin proteins and modifies gluten peptides, through a process called deamidation (selective deamidation of certain glutamine residues). The deamidated gliadin peptide–transglutaminase complexes, presented by antigen with B cells, after binding with HLA DQ2 and/or DQ8 induce the activation of specific gluten-responsive T cells.

The cooperation of activated T and B cells (resulting in the production of antibodies) involves the complex inflammatory process that causes the destruction of the intestinal mucosa [49] and the onset of the disease symptoms. This is the so called ‘adaptive response.’

However, the proteins from wheat, rye and barley can also cause an innate immune response in professional antigen presenting cells (monocytes, macrophages and dendritic cells), thus determining the activation mainly of intraepithelial lymphocytes and intestinal epithelial cells [51–56]. This innate immune response is an immediate reaction and is usually directed against microbial antigens relatively common, but actually even against some constituents (still to be defined) of cereals [57,58].

Furthermore, after the intestinal mucosa stimulation with the peptide p31–49 (and with other peptides) of gliadin epithelial cells, macrophages and dendritic cells secrete IL-15, which

in turn may stimulate the cytotoxic lymphocytes, thus inducing an increased epithelial apoptosis and, consequently, the intestinal barrier permeability.

In fact in CD, the innate immune response seems to favor the development of adaptive immunity to gluten in patients who carry HLA-DQ2 or HLA-DQ8 [53] and, then, IL-15 connects the adaptive immune system with the immune responses innate. This strategic role of IL-15 in the activation of innate and adaptive immunity in CD has been confirmed by numerous investigations [57–61]. Even in the pathogenesis of other autoimmune diseases, the cooperation between adaptive and innate immunity is evident [49]. In patients with T1D, self-reactive CD8 T cells have long been considered responsible for the autoimmune destruction of beta cells but, there is also an increasing evidence that cytokines, such as IFN- γ , IL-1 β and IL-17 released by T cells, are able to directly induce apoptosis of beta cells [62–64].

Furthermore, it has been shown that diabetic patients with HLA-DR genotypes have an increased risk of T-cell reactivity to polypeptides derived from wheat, characterized by IFN- γ secretion and IL-17A secretion [65].

Intestinal barrier function

The intestinal epithelial barrier, with its intercellular tight junctions (TJ), in association with gut-associated lymphoid tissue and neuroendocrine network, controls the balance between tolerance and immunity to nonself-antigens. There are growing but controversial evidences that the increased intestinal permeability plays a pathogenic role in various AI and appears to be an early biologic change that precedes AI and CD onset.

In CD, the interaction of gliadin with the intestinal mucosa seems to lead to the deterioration of the intestinal barrier. The mechanism by which this occurs is not yet clear in all its steps, but it seems that gliadin binds to an intestinal receptor (CXCR3) that leads to the release of zonulin (prehaptoglobin-2) in large quantities. Zonulin is the only physiological modulator of the intercellular TJ permeability described so far and is involved in the trafficking of macromolecules and in the balance between tolerance and immune response to oral antigens. Zonulin is able to create an increased intestinal permeability through a disassembly of the small intestine TJ [66]. This allows a large number of nonself-antigens (including the indigestible fragments of gluten) to reach the underlying lamina propria and elicit an immune response and a subsequent tissue damage in susceptible individuals with the mediation of HLA-DQ-DR molecules; however, the mechanism underlying this involvement is still controversial [67].

Furthermore, dendritic cells, that have tied gliadin, can move from the small intestine and reach the mesenteric lymph nodes and pancreatic islets [68] where they present the gliadin antigens. This event causes the migration of activated T lymphocytes to the target organ (which may be the intestine and/or pancreas), with the consequent contribution to the inflammatory process [69].

Animal studies showed an immunological link between pancreas and gut. T cells, which have been activated in the

gastrointestinal tract, settle in the pancreatic islets. Luminal antigen presentation takes place in the pancreatic lymph nodes. Changes in the gastrointestinal cytokine environment, deriving from infections or abnormal reactivity to intestinal microbiota or food antigens, may alter the T-cells phenotype being hosted in the pancreas.

In effect, there are evidences that an altered intestinal permeability occurs in T1D prior to the onset of complications [70], and this has led to the suggestion that an increased intestinal permeability, due to the alteration in intestinal TJ, is responsible for T1D onset [71,72]. The loss of the intestinal barrier function, due to the increase of zonulin, may be one of the initial steps in T1D pathogenesis, as demonstrated by the fact that oral administration in biobreeding rats of a zonulin inhibitor, known as larazotide acetate (AT1001), seems to block both the formation of autoantibodies and the increase of zonulin-induced intestinal permeability and to also reduce diabetes incidence [72]. Studies performed on humans show that 50% of T1D patients have elevated serum zonulin levels that correlate with the increased intestinal permeability. There are also preliminary evidences indicating that zonulin upregulation precedes the onset of diabetes [73] even if the loss of intestinal barrier function is necessary, but not sufficient for the onset of the autoimmune process.

Also in Phase I of a clinical trial conducted on patients with CD, treatment with AT1001 seems to block the increase in permeability-induced gluten and, therefore, relieve the gastrointestinal symptoms [74]. Although these data have still to be confirmed, they suggest that treatment with the zonulin inhibitor AT1001 [74], as well GFD preventing the activation of zonulin, may block the process of targeting autoimmune intestinal or pancreatic β -cells.

TABLE 1 reports the summary of the main pathogenetic evidences of the association between CD and endocrine AI.

CD & T1D

The association of T1D with other AI in the same subject is relatively common, and it affects the management of the disease in childhood and adolescence. Epidemiological studies reported that CD prevalence in T1D patients ranges from 4 to 11% in comparison to 1% of the general population [75,76].

Screening for CD is now recommended in T1D children at disease onset, annually in the first 4 years of the disease, and every 2 years in the following 6 years [77,78]. Screening is commonly performed by means of IgA antitransglutaminase (confirmed by antiendomysial antibodies) or IgG antitransglutaminase in subjects with IgA deficiency. In the presence of CD-related antibodies positivity, it is mandatory to perform bowel biopsy to confirm diagnosis of CD, even if recent guidelines of the ESPGHAN Society [1] suggest that in clinically evident CD cases, bowel biopsy could be avoided (four major criteria).

A recent paper [79] reported that antitransglutaminase antibodies normalized at 1-year follow-up in 35.4% of children found positive for antitransglutaminase antibodies within 4 months from T1D diagnosis and who continued to take

gluten. A lower proportion of children also showed a concurrent spontaneous normalization of antiendomysial antibodies. At 1-year follow-up, no significant differences were evident in terms of HbA1c levels and growth parameters between T1D children on GFD (for confirmed CD) and T1D children found positive for CD-antibodies who continued gluten-containing diet. The authors suggested that T1D children with mildly elevated antitransglutaminase antibodies at T1D onset should be monitored serologically on a gluten-containing diet, for at least 12 months, rather than undergoing immediately duodenal biopsy.

In most T1D patients, diabetes is the first to be diagnosed, while CD precedes T1D onset in 10–25% of subjects, whose mean age (at CD diagnosis) of clinically symptomatic CD is around 2–3 years, whereas mean age at T1D onset is around 7–8 years [80,81]. CD in T1D patients is commonly asymptomatic or slightly symptomatic, and the diagnosis is reached through the routine screening performed at clinical onset of T1D in 70–80% of cases [82–84].

T1D in untreated & treated CD patients

Kaspers *et al.* [85] reported that subjects with both CD and T1D are younger and showed a lower height standard deviation score (SDS) at T1D onset (-0.49 vs -0.06) than children with isolated T1D, suggesting that the poor height growth may represent a sign of a previously undiagnosed CD. In addition, body mass index SDS significantly differs between the two groups (0.22 vs 0.47). In two separate studies, Cerutti *et al.* and Valerio *et al.* demonstrated the presence of a higher risk of developing both diseases in girls than in boys. They also reported that both the younger age at T1D onset and the concurrent thyroid disorder were variables independently associated with the risk of suffering from both T1D and CD.

Cerutti *et al.* [80], in a large group of Italian children, confirmed the higher risk of both diseases in girls (odds ratio [OR]: 1.93). Logistic regression analysis showed that the younger age at T1D onset, female gender and a concurrent thyroid disorder are variables independently associated with the risk of having both T1D and CD. In children younger than 4 years at T1D onset, the risk of developing both diseases was higher if compared with children older than 9 years.

Valerio *et al.* [81] identified 32 CD subjects in a group of 383 T1D patients (8.3%). Gender distribution, age at T1D onset, prevalence of ketoacidosis at T1D, clinical presentation and prevalence of other AI were compared in patients with CD diagnosed before (Group A: 8 subjects) or after (Group B: 24 subjects) T1D onset. Patients of Group A had presented clinical symptoms suggestive of CD, whereas patients of Group B were identified by the routine screening for CD as T1D subjects. Group C (351 subjects) included diabetic patients without CD. The proportion of girls was significantly higher in Groups A and B compared with Group C ($p = 0.013$). A higher prevalence of ketoacidosis ($p = 0.009$) and other AI ($p = 0.001$) and a lower age at diabetes onset was evident in Group A.

Table 1. Main pathogenetic evidences of the association between celiac disease and endocrine autoimmune diseases.

Factors	Pathogenetic agent/mechanism	Ref.
Genetics	HLA DR3-DQ2 and DR4-DQ8 haplotypes common in many autoimmune diseases Many CD risk loci are related to the immune response (particularly to B- and T-cell function)	[12,13] [11]
<i>Environment</i>		
Gluten	The exposure of an immature immune system to gliadin in susceptible individuals seems to be a prominent cofactor in modifying the immunological response earlier in life, thus predisposing susceptible individuals, not only to overt CD, but also to AI	[5,9]
Breastfeeding	Breastfeeding is thought to delay or reduce the risk of developing CD. This protective effect seems to be due to the different pattern of intestinal microbiota in breastfed infants	[20,21,31]
Intestinal microbiota	The different composition of the intestinal microbiota in CD patients, untreated and treated by GFD. Human studies about alterations in the composition of the intestinal microbiota and development of T1D. Animal models support the hypothesis that intestinal bacteria may be involved in the early onset of T1D. Studies that link the gut and Hashimoto's thyroiditis (just indirect and weak evidence for such a link)	[26–39]
Infectious diseases	Infections can influence the host's immune tolerance by different mechanisms: Polyclonal lymphocyte activation Increased immunogenicity of organ autoantigens secondary to infection-mediated inflammation Antigen mimicry molecular mechanisms	[40–42]
Immunology	As for AI, both adaptive and innate immune responses are involved in the pathogenesis of CD	[55]
Gut barrier function	There is a growing evidence that increased intestinal permeability plays a pathogenic role in various AI including CD and T1D. Actually, in several autoimmune diseases there is a loss of intestinal barrier function. The increased intestinal permeability appears to be an early biologic change that precedes the onset of AI and CD	[66–74]

AI: Autoimmune diseases; CD: Celiac disease; GFD: Gluten-free diet; T1D: Type 1 diabetes.

Norris *et al.* [86] analyzed 1183 children genetically at risk of developing T1D depending on the age of the first exposure of cereal in the infant diet. Children first exposed to cereals before 3 months of age and those exposed after 7 months of age showed a higher hazard ratio (HR) of developing β -cell autoantibodies, thus indicating that the time of gluten introduction in the diet might impact the risk of developing T1D later in life. A similar result was also reported in the BABYDIAB Study: the early introduction of gluten in the infant diet resulted in an increased appearance of islet-related autoantibodies, but it did not influence the appearance of CD serological markers.

Such study [87] was performed on 1,610 infants born to T1D parents. Blood samples were taken at birth, 9 months, 2, 5 and 8 years of age. Breastfeeding and food supplementation data were collected by questionnaires and family interviews. Aim of the study was to analyze the development of β -cell autoantibodies against insulin (IAA), glutamic acid decarboxylase (GAD) or insulin-associated protein 2 (IA-2) and its correlation with the diet. A reduced total or exclusive breastfeeding duration did not significantly affect the risk of developing β -cell autoantibodies. Food supplementation with gluten-containing foods before 3 months of age was associated with a significantly higher risk of β -cell autoimmunity (adjusted HR: 4.0; 95% CI: 1.4–11.5), in comparison with children receiving exclusive breast milk after the first 3 months of life. Four out of the 17 children, who had received gluten foods before the

age of 3 months, developed β -cell autoantibodies, all of them at high risk of DRB1*03/04,DQB1*0302 genotype. The early gluten exposure did not significantly augment, whereas the risk of developing CD autoantibodies definitely increased.

Ventura *et al.* [88] tested 90 CD patients (mean age at diagnosis 10.1 years) for serum levels of EMA, Islet cell antibodies (ICA), GAD autoantibodies, IAA at CD diagnosis and after 6, 12 and 24 months of GFD. At the time of CD diagnosis, all subjects were found positive for EMA and 11 were positive for at least one islet-related antibody. By the end of the second year of follow-up on GFD, all patients resulted negative for both EMA and islet-related antibody, thus demonstrating that GFD might influence the appearance of serological markers of risk proving that the patient is at risk of developing T1D. In line with this latter study, Toscano *et al.* [89] found that gluten challenge determines a significant development of GAD, ICA and IAA in CD patients compared with CD patients on a GFD [88]. In both these studies, islet-related autoantibodies did not show any association with the progression toward T1D. On the basis of these results what about the role of GFD in the prevention of T1D?

GFD & T1D prevention

Primary prevention

The BABYDIET trial [90] has been designed to analyze whether the delay of the complementary gluten food beginning is able

to prevent development of β -cell autoimmunity in newborns with a high risk of developing T1D. For this survey, the authors enrolled children younger than 2 months of age, not yet exposed to gluten, who had at least two first-degree relatives with T1D or one first-degree relative with T1D plus an HLA genotype at risk for T1D. One hundred and fifty children were randomly assigned to a first gluten exposure at 6 months of age as routinely recommended (control group) or at 12 months of age (late-exposure group). All were followed-up every 3 months until the age of 3 years and yearly thereafter. Preliminary results [90] have shown that during the first 3 years of age, weight, height and prevalence of autoantibodies to transglutaminase were similar in children of the two groups. Eleven children in the control group and 13 children in the late-exposure group developed islet-related autoantibodies. Six children developed autoantibodies before their first reported gluten exposure. HR for developing β -cell autoantibodies 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 substantially reduce the risk of β -cell autoimmunity activation in genetically susceptible children. The increased gut permeability related to gluten exposure cannot be identified as the trigger of β -cell autoimmunity, because no child was exposed to dietary gluten prior to the study, and six children were found positive to the autoantibodies even before the first gluten exposure. It could be argued that gluten exposure, with the consequent increase of gut permeability and intestinal inflammation does not represent the primary trigger of autoimmunity, but one of the environmental factors contributing to the amplification of the autoimmune process directed against β -cells. Recently, the DAISY group [91] analyzed the association between reported illnesses during infancy and later development of β -cell autoimmunity. Complete illness reports through the first 9 months of age were collected for 1,729 children: 1,174 without a family history of T1D and 555 with a first-degree relative affected by T1D. Children were examined at 9, 15 and 24 months of age and yearly thereafter. Blood samples were drawn at each visit and tested for β -cell antibodies. Persistent β -cell autoimmunity was defined as positive IAA, GAD and IA2 antibodies at least in two consecutive visits. During the course of the study, 124 children developed persistent β -cell antibodies. The number of illnesses reported during the first 9 months of life did not vary between those children who did not develop antibodies versus those who developed antibodies for gastrointestinal disorders, respiratory diseases, febrile episodes or upper respiratory symptoms. Having a first-degree relative with T1D and carrying a high-risk HLA genotype were associated with a higher risk of developing β -cell autoimmunity. Exposure to wheat or barley either early (0–3 months) or late (>7 months) was associated with an increased risk of developing β -cell autoimmunity compared with exposure at 4–6 months of age. The only significant interaction between gluten introduction and infancy illnesses was the association with gastrointestinal infections, that differed

by the timing of introduction of food containing gluten. Each gastrointestinal illness was associated with a 37% increased risk of antibody development among children exposed to wheat or barley before 4 months of age, and a 12% increased risk of autoimmunity among children not exposed to wheat or barley until 7 months of age. Among children with wheat or barley introduction between 4 and 6 months of age, no association between gastrointestinal illnesses and persistent antibodies was found. The authors hypothesized that infections affecting the gastrointestinal system tract may increase the risk of β -cell autoimmunity in the presence of a co-existing inflammation induced by diet.

Secondary prevention

It is also well known that T1D and other AI are less common in patients with CD diagnosed at a younger age, suggesting that the early elimination of gluten may prevent the manifestation of other autoimmune conditions [5,6,78,82,83].

A trial consisting of 6 months of GFD followed by additional 6 months of normal gluten-containing diet was conducted on 17 first-degree relatives with at least 2 β -cell antibodies (GAD, IA2 and IAA). The effect of treatment was measured as autoantibody titers and acute insulin response to intravenous glucose tolerance test [92]. Two subjects dropped out for poor compliance to diet restrictions. Three of the remaining 15 subjects developed diabetes. Autoantibody titers did not show significant changes after 6 months of GFD and after the return to a normal diet. Acute insulin response to intravenous glucose tolerance test results significantly increased in 12/14 subjects after the first 6 months of gluten deprivation, and decreased in 10/13 subjects on a normal diet during the following 6 months. In any case, the overall change observed was small. Considering the nine subjects with insulin secretion <1st centile at the beginning of the study, one developed diabetes and seven showed an increased insulin secretion (>3 centile only in two subjects) during GFD. During the following 6 months of normal diet, one subject developed diabetes, and all the others showed a decline of insulin secretion, one of them developed diabetes soon after the end of the study. Insulin sensitivity [analyzed by homeostasis model assessment for insulin resistance (HOMA-IR)] slightly improved after GFD and subsequently decreased after 6 months of normal diet. Even if this survey did not include an untreated control group, the changes in insulin secretion after IVGTT were considered to be related to the treatment, because the trend was reversed after the restarting of the gluten-containing diet. Furthermore, the authors noted that the trend observed for insulin secretion was in line with that of insulin sensitivity as assessed by HOMA-IR. The mechanism by which gluten withdrawal could improve insulin secretion and insulin sensitivity is quite unclear. The characteristics of the carbohydrates in the GFD may have influenced insulin response to glucose, but it is also possible that autoimmune β -cell destruction may have been improved without any considerable effect on autoantibody titers [92].

Table 2. Gluten exposure and development of Type 1 diabetes: summary of the main evidences.

Study (year)	Is the lower age at CD diagnosis protective?	Is the GFD protective toward the autoantibodies appearance?	Ref.
Ventura <i>et al.</i> (2000)	NE	Yes	[88]
Toscano <i>et al.</i> (2000)	Not	Yes	[89]
Valerio <i>et al.</i> (2002)	Not	NE	[81]
Norris <i>et al.</i> (2003)	NE	Yes	[86]
Kaspers <i>et al.</i> (2004)	Not	NE	[85]
Cerutti <i>et al.</i> (2004)	Not	NE	[80]
Fuechtenbusch <i>et al.</i> (2004)	NE	Not	[93]
Hummel <i>et al.</i> (2011)	NE	Not	[90]

CD: Celiac disease; GFD: Gluten-free diet; NE: Not evaluable.

Fuechtenbusch *et al.* [93] carried out a pilot study with the aim of clarifying whether dietary gluten elimination can influence β -cell autoimmunity. Seven first-degree relatives of patients with T1D, ranging from 1.2 to 5.9 years of age with elevated levels of islet-related autoantibodies were recruited and administered GFD for 12 months, followed by a normal gluten-containing diet for a further 12 months. IAA, GAD, IA-2 and antitransglutaminase antibodies were measured prior to the diet and every 3 months. During the follow-up, only one subject showed significant decreases of antibody levels in the GFD period. Both this subject and another 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. The main clinical studies on the association between CD and T1D are summarized in TABLE 2.

Celiac disease & thyroid autoimmunity

CD patients, aged <18 years, show a wide variety of TA prevalence without thyroid disease ranging from 12 to 26% [88–89,94–98]. On the contrary, the prevalence of biopsy-proven CD in TA patients seems to reach up to 7% [99]. Therefore, regular monitoring for CD in TA patients is advised [1]. There are no current recommendations for TA screening in CD patients. Various screening strategies have been considered advisable in the following clinical conditions: poor growth, weight loss or other signs or symptoms of hypo- or hyperthyroidism [95]; at CD diagnosis and at follow-up evaluation [94]; at CD diagnosis and only in CD who have positive antithyroid antibody titers and at follow-up [95].

TA in untreated & treated CD patients

Several studies have investigated the prevalence of TA in CD patients and the role of GFD in modulating TA. Ansaldi *et al.* [94], in a multicenter Italian study, investigated 256 CD patients on GFD, 87 untreated CD patients and 230 controls.

TA was found in 26.2% of CD patients and in 10% of controls. However, prevalence of TA did not show differences between treated and untreated CD patients. In the study by Meloni *et al.* [97], TA prevalence in untreated CD patients was 10.5% significantly higher when compared with the Sardinian pediatric control subjects (2.92%).

In several studies, adherence to GFD has been considered as a factor that might influence TA prevalence in CD patients. Oderda *et al.* [96] reported a TA prevalence of 14.6% in untreated CD children. Follow-up of TA in these patients on GFD demonstrated a disappearance of TA in one-third of them. Furthermore, the majority of children with TA was older than 6 years at the time of CD diagnosis, thus suggesting that the duration of gluten exposure might play a role in TA development.

Toscano *et al.* [89] studied TA in 44 CD patients. They were grouped according to their diet regimen: 25 adolescents were on GFD, whereas 19 on a gluten-containing diet to make the gluten challenge age was similar in the two groups. TA was investigated by evaluating antibodies against peroxidase (ATPO) and thyroglobulin (ATG). In 9 out of 44 subjects (20.4%), at least one antibody directed against thyroid tissue was positive. With respect to dietary habits, the presence of one or more antibodies was significantly higher in untreated (52.6%) than in treated patients (20%). The absolute values of ATG and ATPO titers were higher in treated than in untreated patients, reaching a statistical significance only for ATPO. A condition of hypothyroidism was present in 8.1% patients with TA.

Ventura *et al.* reported a prevalence of TPO antibodies in 14.4% of patients at CD diagnosis, which declines up to 2.2% over a period of 24 months on a GFD. TA was not associated with thyroid function abnormalities both at diagnosis and follow-up [88].

TA in treated CD patients

The surveys performed on treated patients lead to understanding the role of the so called 'actual gluten exposure,' on

Table 3. Gluten exposure and development of thyroid autoimmunity: summary of the main evidences.

Study (year)	Is the lower age at CD diagnosis protective?	Is GFD protective toward TA development?	TA+ impaired thyroid function (%) [†]	Ref.
Ventura <i>et al.</i> (2000)	NE	Yes	NE	[88]
Toscano <i>et al.</i> (2000)	Not	Yes	25	[89]
Oderda <i>et al.</i> (2002)	Yes	Yes	17	[96]
Ansaldi <i>et al.</i> (2003)	NE	Not	8.1	[94]
Meloni <i>et al.</i> (2009)	Yes	Not	18	[97]
Cassio <i>et al.</i> (2010)	Not	Not	26	[95]
Diamanti <i>et al.</i> (2011)	Not	Not	9.2	[98]

[†]Presence of clinical or subclinical hypothyroidism (subclinical hypothyroidism is considered if TSH was from 4 to 10 µIU/ml and fT3 and fT4 were normal).

CD: Celiac disease; GFD: Gluten-free diet; NE: Not evaluable; TA: Thyroid autoimmunity.

TA development. This was the main objective of a survey conducted in our center between January and December 2005 [98]. During this study period, 558 patients were prospectively followed-up until December 2007. At the study entry, the median age of patients with CD was 10.9 years (range 1.9–24) and median disease duration was 2.8 years (range 1–14.8). At enrollment, the percentage of subjects with TA was not significantly higher in patients with CD (10%) than in controls (8.2%). Therefore, in this study, unlike the previously reported Italian experiences [94,97], we did not find a significant difference in TA prevalence between CD patients and controls. The markers of thyroid function, as well as the results of thyroid ultrasound, were also similar in patients with CD and controls. After 2 years of follow-up, CD patients with TA increased to 12% demonstrating that the strict GFD did not protect them from TA development. Our data also showed that TA course in CD subjects is quite slow: hypothyroidism requiring hormone replacement therapy was found in 2% of patients with TA at study entry and in a further 7% after 2 years of follow-up. Therefore, 93% of patients with TA were still euthyroid for at least 2 years after the original diagnosis.

Similar results were reported by Cassio *et al.* [95] who reviewed the medical records of 135 consecutive patients diagnosed with CD at June 1990 and December 2004 (age at diagnosis, 5.7 ± 3.9 years) and followed-up for 8.9 ± 4.0 years (range 3–17 years) on a GFD. At diagnosis, negative antithyroid antibody titers were observed in 119 of 135 patients (88%) and positive antibody titers in 16 of 135 patients (12%). At the final assessment, negative antithyroid antibody titers were observed in 104 of 135 patients (77%) and positive antibody titers in 31 of 135 patients (23%). This study suggests that GFD is not protective toward the development of TA. Similarly to our study [98], Cassio *et al.* [95] found that TA in children and adolescents with CD shows a very low cytotoxic activity with a tendency to develop overt hypothyroidism only in 12% of cases.

TABLE 3 shows the main clinical evidences regarding the association between CD and TA.

CD & other associated endocrine AI

Other endocrine AI associated with CD are AD [100–103] the primary hypoparathyroidism [103] and the autoimmune polyglandular syndrome (APS) [103], a rare endocrine disorder that includes a combination of at least two endocrine AI. Idiopathic autoimmune hypoparathyroidism and AD in childhood are commonly associated with hypofunction of other endocrine organs in the APS [3], and the association with CD is also seen in patients with APS rather than isolated AD or hypoparathyroidism [103].

Malabsorption is commonly associated with Type 1 APS and it is due to CD, as confirmed by the multicenter Italian study carried out by Betterle *et al.* This survey evaluated 109 patients affected by AD (age range 11–87 years), including 8 with Type 1 APS (at least two present among chronic candidiasis, chronic hypoparathyroidism and AD), 61 with Type 2 APS (AD, TA and/or T1D but AD always present) and 40 with isolated AD. The prevalence of CD in the overall groups was 5.5% (12.5% of Type 1 APS patients, 6.7% of Type 2 APS patients, 2.5% of isolated AD patients, respectively). All cases of associated CD were observed in adults. These results are in part confirmed by the report of Elfstrom *et al.* [101], the largest study of the co-occurrence of AD and CD. In this survey, individuals with CD were at an 11-fold increased risk of subsequent AD, whereas individuals with AD were at an eightfold increased risk of developing later CD. The positive association between CD and AD was seen both before and after diagnosis of CD, thus excluding the protective effect of gluten withdrawal toward the development of AD. High-risk estimates for AD were seen both in childhood and adulthood CD; however, AD incidence in CD children was 3.5/100,000 person-years versus 19.9 in adults. O'Leary *et al.* [102] also found 5 cases of CD (12.2%), all diagnosed in adults, among 41 AD patients, over a follow-up period of 30-years.

Expert commentary & five-year view

During the last 15 years, there have been dramatic advances in the knowledge of the association between CD and other AI,

that induce to revise the concept regarding the potential impact of gluten exposure on AI development and, as a consequence, the way of a clinically useful screening strategy.

The first reports supported the hypothesis that AI could be 'gluten-dependent.' Indeed, an early GFD resulted able to induce, in a short-term follow-up, the disappearance of T1D and thyroid-related autoantibodies [88,96]. Assuming that the organ-specific autoantibodies could play either a predictive pathogenic role in developing AI, the authors concluded that GFD might be able to protect against the development of other AI [88]. Nevertheless, the available evidences seem to confute these conclusions. In fact, GFD has not proven to be able to prevent the development of T1D in infants with a high T1D risk [93] nor in first-degree relatives of patients with T1D presenting elevated levels of β -cell autoantibodies [93]. Furthermore, recently the concomitant development of T1D and CD has been observed in young subjects, therefore showing that the length of gluten exposure before CD diagnosis, does not impact the co-occurrence of the two diseases [80]. In line with what was observed for T1D, also the development of TA seems not to be influenced by gluten exposure, as showed by some surveys with a long-term follow-up [95,97,98]. It is possible that the disappearance of the autoantibodies seen over a short period of observation [88] could be due to their physiologic fluctuation.

The screening strategy for the co-occurrence of CD and endocrine AI deserves some further considerations. In CD, the available screening tests (antitransglutaminase antibodies) early recognize a potential intestinal damage induced by the gluten and its removal leads to the recovery of the intestinal lesions. Unlike the antitransglutaminase, in the endocrine AI, recognizing the autoantibodies often takes many years before the irreversible damage of the target organ, the symptoms and the need of a specific treatment occur. These assumptions are confirmed by the reports on the long-term follow-up of TA in

CD patients: in treated CD patients with TA, indeed, an impaired thyroid function develops in about 13% of all cases, over a mean 8-year follow-up period [95,97]. Therefore, whether the systematic screening for CD in T1D, TA and AD is clinically useful and clearly indicated, in CD patients, the screening for the potentially associated endocrine AI is not clearly indicated. Additionally, based on the recent clinical advances, it seems to be not cost-effective, at least until effective preventive strategies for the AI are available.

In conclusion, the recent clinical advances suggest that gluten exposure cannot explain the complex relationship between CD and AI; in particular, gluten withdrawal seems not clearly related to the disappearance or reduced incidence of endocrine AI. Further pathogenetic factors, in particular the loss of the intestinal barrier function and the common genetic background, seem to be involved in the co-occurrence of CD and endocrine AI [12,14,82–85]. Regarding the screening strategy, the systematic serological detection for CD in T1D, TA and AD is clinically useful and always indicated. If a patient with endocrine AI is seropositive and asymptomatic for CD and does not show conclusive evidence of CD after the extended evaluation of biopsies (potential CD), then such a patient should be followed-up on a normal gluten-containing diet and re-evaluated at regular intervals [101].

Conversely, in CD patients screening for the potentially associated endocrine AI is not clearly suggested. Due to the fact that in many cases, there is an extended latency between appearance of autoantibodies and development of the impaired organ function, the screening for endocrine AI in CD patients, does not seem to be clinically relevant nor cost-effective. Nevertheless, TA, that is very frequent in the general population and in CD patients, can occasionally be evaluated during the follow-up, especially if some clinical symptoms are suggestive of thyroid impairment.

Key issues

- Celiac disease (CD) is a life-long inflammatory condition of the gut that occurs in genetically susceptible individuals associated with several endocrine autoimmune diseases (AI).
- In pediatric and young patients, Type 1 diabetes (T1D) and thyroid autoimmunity (TA) are often associated with CD.
- Epidemiological studies report that the prevalence of CD ranges from 4 to 11% in T1D patients and it is about 7% in TA patients.
- The coexistence of CD and endocrine AI involves a complex interplay between genetic aspects, the environment, immunologic aspects and the intestinal barrier function.
- The first reports supported the hypothesis that the AI could be 'gluten-dependent' because the gluten withdrawal, in the short-term follow-up, has resulted in the disappearance of T1D and TA-related autoantibodies.
- Recent surveys show that the gluten withdrawal does not prevent the development of T1D in newborn infants with a high T1D risk nor in first-degree relatives of T1D patients with elevated levels of β -cell autoantibodies and it does not impact the trend of TA in long-term follow-up. Overall, the recent clinical advances suggest that gluten exposure cannot explain the complex relationship between CD and AI, but other pathogenetic factors, in particular the loss of the intestinal barrier function and the common genetic background, are involved.
- The systematic screening for CD in T1D and TA is clinically useful and clearly indicated.
- The systematic screening for the potentially associated endocrine AI in CD patients is not clearly indicated and, based on the recent clinical advances, it seems, neither clinically relevant nor cost-effective, at least until effective preventive strategies for these AI become available.

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