Coeliac disease and gluten-related disorders in childhood

Sabine L. Vriezinga, Joachim J. Schweizer, Frits Koning and M. Luisa Mearin

Abstract | Gluten-related disorders such as coeliac disease, wheat allergy and noncoeliac gluten sensitivity are increasingly being diagnosed in children. Coeliac disease occurs frequently, affecting 1–3% of the Western population. The condition manifests at a very young age, more so in girls, and is related to the HLA genotype. Coeliac disease might be considered a public health problem and, as primary prevention is not possible, the debate on mass screening should be reopened. Wheat proteins, including gluten, are responsible for one of the most common food allergies in children: wheat allergy. Unlike coeliac disease and wheat allergy, noncoeliac gluten sensitivity is an unclear and controversial entity. These three gluten-related disorders are treated with a gluten-free diet. In coeliac disease, the diet should be strictly followed, whereas wheat allergy only requires wheat elimination and in noncoeliac gluten sensitivity occasional trials of gluten reintroduction can be done. A good diagnostic work-up is important for gluten-related disorders in childhood to avoid unnecessary restrictive diets in children. In this Review, we provide an overview of the pathogenesis, diagnosis and management of the most common gluten-related disorders in children.

Vriezinga, S. L. *et al. Nat. Rev. Gastroenterol. Hepatol.* advance online publication 23 June 2015; doi:10.1038/nrgastro.2015.98

Introduction

Gluten is a group of storage proteins in cereals such as wheat (gliadins), rye (secalins), and barley (hordeins). A few years ago, the spectrum of gluten-related disorders in children was restricted to coeliac disease and wheat allergy but, nowadays, the new entity referred to as noncoeliac gluten sensitivity (NCGS) is gaining recognition.[1] Unlike coeliac disease and wheat allergy, NCGS is an unclear and controversial entity (Table 1). The aim of this Review is to provide an overview of the pathogenesis, diagnosis and management of the most common gluten-related disorders in children (Figure 1).

Coeliac disease

Coeliac disease is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals, characterized by the presence of a variable combination of gluten-dependent clinical manifestations, coeliac-disease-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy.[2] In coeliac disease, gluten peptides activate T cells that mediate a self-perpetuating inflammatory process. This process leads to mucosal damage of the small bowel and other organs, producing symptoms ranging from malabsorption with diarrhoea, abdominal distension and weight loss, to nonspecific signs and symptoms such as fatigue, osteoporosis or iron deficiency anaemia (Box 1).[2]

Childhood coeliac disease is a common disorder, with a 1–3% prevalence in the general Western population that includes the USA, corresponding to about 5 million people in the European community, the highest frequency of which reside in Sweden.[3] Therefore, coeliac disease might be considered as a public health problem in both Europe and the USA.[2,4] Coeliac disease is also frequent in South America,[5,6] the Middle East, North Africa and India, where wheat has been the major staple food for centuries, but rare among native Africans, Japanese and Chinese people.[7–9] A high index of suspicion for coeliac disease should be maintained in all developing countries in children who present with chronic diarrhoea and malnutrition.[10] Despite the increasing numbers of positive diagnoses for coeliac disease, the condition is frequently unrecognized, possibly due to its variable clinical presentation and symptoms,[11,12] such that for every one child diagnosed with coeliac disease, there are seven who remain undiagnosed.[13–15] Coeliac disease can also affect extraintestinal organs. In fact, nongastrointestinal manifestations are now more common in children than before, possibly because of a greater awareness of symptom diversity.[2,16] Coeliac disease can occur at any age; however, prospective screening studies demonstrate that the disease appears at a very young age (<3 years old) in children with a first-degree relative with coeliac disease.[17] In children with coeliac disease and a family history of coeliac disease, 50% had developed the disease by age 3 years.[17] By 3 years of age, coeliac disease has a female preponderance of 2–3:1, with an incidence of 7.2% in girls with a positive family history versus 3.4% in boys.[17] Generally, first-degree family members of patients with coeliac disease have an increased risk of the disease (ranging from 2–20%), depending on gender and HLA haplotype.[17] Patients with other autoimmune diseases, including type 1 diabetes mellitus, autoimmune

Department of Paediatrics (**S.L.V.**, **J.J.S.**, **M.L.M.**) and Department of Immunohematology and Blood Transfusion (**F.K.**), Leiden University Medical Centre, Albinusdreef 2/PO 9600, 2300 RC Leiden, Netherlands.

Correspondence to: M.L.M.
l.mearin@lumc.nl

Competing interests
The authors declare no competing interests.

Key points

- The fundamental step in diagnosing coeliac disease is awareness of symptom diversity; anti-transglutaminase antibodies are very specific for the diagnosis and, in children, duodenal biopsies can sometimes be omitted
- Prospective studies show that coeliac disease manifests at a young age, more often in girls, and is related to the HLA genotype, but not the timing of gluten introduction or breastfeeding
- Wheat allergy is one of the most common food allergies in children beginning in early childhood; it is less common in adolescents and adults; most children outgrow wheat allergy by 12 years
- Noncoeliac gluten sensitivity is a clinical condition in which symptoms are triggered by gluten ingestion in the absence of coeliac disease and wheat allergy
- No biological markers exist for noncoeliac gluten sensitivity, exclusion of coeliac disease and of wheat allergy is the most important diagnostic step
- Once a gluten-related disorder is diagnosed, children should be referred to a paediatric dietitian for in-depth guidance about the necessary dietary treatment

thyroid disease, or patients with selective IgA deficiency, as well as those with Down syndrome, Turner syndrome and Williams syndrome, also have an increased risk of developing coeliac disease (Box 2).[2]

Pathogenesis

Virtually all patients with coeliac disease express the HLA-class II molecules HLA-DQ2 and/or HLA-DQ8, and gluten-specific HLA-DQ2/8-restricted $CD4^+$ T cells can be isolated from their small bowel mucosa.[18] Wheat gluten is composed of different gliadins and glutenins; immunogenic epitopes have been identified in all these proteins.[19–27] Some of these epitopes found in the α-gliadins and ω-gliadins, barley hordeins and rye secalins, are more immunodominant as they trigger T-cell responses in almost all patients.[19–21,23,24] Typically, these epitopes are proline-rich, which render them resistant to enzymatic degradation.[21] Moreover, they contain an amino acid sequence wherein the glutamine (Q) can be modified into glutamic acid (E) by the enzyme transglutaminase type 2 (TG2), thereby introducing a negative charge required for high-affinity binding to HLA-DQ2 and recognition by $CD4^+$ T cells (Figure 2).[28–30] In coeliac disease, there is a strong HLA-DQ gene-dose effect: HLA-DQ2 homozygous individuals have a much higher risk of developing coeliac disease than those who are heterozygous.[31] This effect correlates with stronger T-cell responses to gluten peptides when presented by HLA-DQ2 homozygous cells, indicating that the level of gluten presentation influences the risk of disease development.[32] Interestingly, there are no indications for an HLA-DQ2 gene-dose effect once the disease has developed because the symptoms and severity of intestinal lesions in childhood coeliac disease are similar in HLA-DQ2 homozygous and heterozygous individuals.[33] Apparently, once tolerance is lost, the level of antigen presentation in the intestine is sufficient to sustain the inflammatory gluten-specific $CD4^+$ T-cell response.[32,34] This process might relate to the local production of IFN-γ by these $CD4^+$ T cells, widely known to enhance HLA expression on antigen-presenting cells.[32]

After the disease-causing gluten-specific T-cell response in the lamina propria, major changes occur in the composition, size and activation state of the intraepithelial lymphocyte (IEL) compartment in patients with coeliac disease.[35] Normally, IELs are found scattered throughout the intestinal epithelium and are located at the basolateral side of the epithelial cell layer. Although the majority of IELs are $CD8^+$αβ T-cell receptor $(TCR)^+$ T cells, higher numbers of both $CD8^+$αβTCR^+ and TCRγδ$^+$ T cells are found in patients with coeliac disease than in healthy individuals (Figure 2).[34,36] Moreover, IELs are found at the tip of the villi in coeliac disease, indicating a redistribution of the IELs in the epithelium, not observed in healthy individuals.[37] Although the importance of the increased number of TCRγδ$^+$ T cells in coeliac disease remains unclear, $CD8^+$αβTCR^+ T cells gain a natural-killer-like phenotype, suggesting that they might be involved in the epithelial cell killing and remodelling observed in active coeliac disease.[38] IL-15 has a key role in coeliac disease as it is overexpressed by the epithelial cells and can directly activate adjacent IELs.[38,39] In addition, it is feasible that cytokines released by adaptive T cells in the lamina propria, such as IL-2 and IL-21, can reach the epithelial compartment and contribute to the activation of IELs. Thus, the changes in the epithelial compartment could be secondary to the activation of $CD4^+$ gluten-reactive T cells in the lamina propria. Alternatively, it is possible that intrinsic aberrations in the epithelial layer cause the observed characteristic changes. Strikingly, the number of $CD8^+$αβTCR^+ T cells normalizes but the numbers of TCRγδ$^+$ T cells remain elevated and do not seem to have a pathogenic role upon initiation of a gluten-free diet (GFD) but, rather, might be required to maintain epithelial homeostasis.[41]

Table 1 | Characteristics of coeliac disease, wheat allergy and noncoeliac gluten sensitivity

Characteristics	Coeliac disease	Wheat allergy	Noncoeliac gluten sensitivity
Disease frequency	1–3%	0.1%	Unknown; estimated 0.5–6% in adults
Trigger	Gluten peptides	Wheat	Unknown; gluten, FODMAPs and α-amylase trypsin inhibitors have been implicated
Pathogenesis	Autoimmune	Allergic	Unknown
Genetics	HLA-DQ2 and HLA-DQ8	Atopic constitution	Unknown; over-representation of HLA-DQ2?
Biomarkers	TG2A, EMA, DGPA	Wheat-specific IgE	None
Treatment	Strict, life-long gluten-free diet	Wheat-free diet	Gluten-free diet, reintroduction of gluten possible

Abbreviations: DGPA, anti-deaminated gliadin peptide antibody; EMA, anti-endomysium antibody; FODMAP, fermentable oligosaccharide, disaccharide, monosaccharide and polyol; TG2A, anti-transglutaminase type 2 antibody.

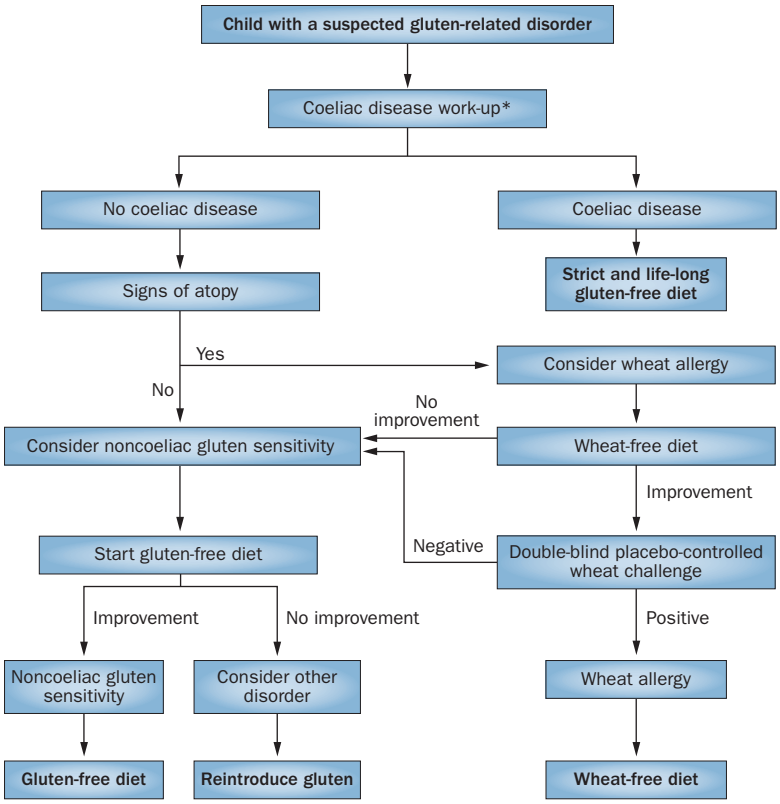

Figure 1 | Flow-chart of the diagnostic process in a child with a suspected gluten-related disorder. *See Husby *et al.*[2] and/or Figure 3 and 4.

Box 1 | Symptoms of childhood coeliac disease

Gastrointestinal
- Diarrhoea
- Anorexia
- Vomiting
- Growth retardation, weight loss
- Chronic abdominal pain
- Chronic constipation
- Distended abdomen

Extraintestinal
- Chronic fatigue
- Iron deficiency anaemia
- Macrocyticanaemia (folic acid and/or vitamin B_{12} deficiency)
- Dermatitis herpetiformis
- Dental enamel hypoplasia
- Recurrent aphthous mouth ulceration
- Arthritis
- Arthralgia
- Osteopenia or osteoporosis
- Bone fractures
- Mildly elevated levels of AST and ALT
- Short stature
- Late puberty
- Cerebellar ataxia
- Recurring headaches
- Peripheral neuropathy
- Seizures
- Anxiety
- Depression

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Next to adaptive IELs, the epithelium also has at least four subsets of innate lymphocytes.[41] Little is known about the function of these innate lymphoid subsets that are present in high numbers in children, especially in young children, but far less so in healthy and affected adults with coeliac disease. One of these subsets bears a resemblance to the IFN-γ-secreting type 1 innate lymphoid cell (ILC1), the innate homologue of $CD4^+$ T_H1 helper cells whereas another, the lineage-negative IEL, has a distinct phenotype responsive to IL-15.[41] The latter is the likely precursor to the aberrant monoclonally expanded cells in patients with refractory coeliac disease type 2: a premalignant condition unresponsive to a GFD that is very rare in children.[41] In addition to these genetic and immunological factors, environmental factors including elective Caesarean section, perinatal and childhood infections, the use of antibiotics and PPIs, and changes in the microbiota might have a role in the pathogenesis of coeliac disease.[42,43]

Diagnosis

The key to the diagnosis of coeliac disease in children is a high degree of awareness of its wide spectrum of symptoms (Table 1 and Box 1). Coeliac disease is thereby diagnosed through a combination of detection of coeliac-disease-specific autoantibodies, HLA-DQ typing and small bowel biopsies that are performed while the patient is on a gluten-containing diet.[2]

Clinical presentation

The clinical presentation of childhood coeliac disease is partially age-dependent. Very young children (<3 years) present more commonly with chronic diarrhoea, abdominal distension and growth retardation whereas older children and adolescents (≤18 years) present with milder gastrointestinal symptoms such as recurrent abdominal pain, vomiting or constipation. Extraintestinal symptoms such as arthritis, neurological symptoms and anaemia are also frequent.[2,44] In addition, coeliac disease can be asymptomatic, as was the case for 43% of the children identified by family screening.[17]

Autoantibodies

Specific coeliac disease autoantibodies are detected in the serum against TG2 (TG2A), endomysium (EMA), and deamidated gliadin peptides (DGPA).[2] In the case of severe histological small bowel alterations, IgA TG2A and EMA have high sensitivities (98% and 90%, respectively) and specificities (97% and 98%, respectively), which are also observed in very young children.[17,45] In those with less severe intestinal damage, these specificity and sensitivity values are lower.[45] Total IgA measurement is also important because coeliac disease is associated with selective IgA deficiency.[46] In IgA deficiency, IgG coeliac disease antibodies should be determined, among which, IgG DGPA is most suitable, with diagnostic values comparable to IgA TG2A.[45]

HLA-typing

HLA-typing is not advised in the routine diagnosis of coeliac disease because 40% of the general European and

Box 2 | Childhood coeliac disease in other conditions

- Type I diabetes mellitus: 3–12%
- Selective IgA deficiency: 2–8%
- Autoimmune thyroiditis: ≤7%
- Down, Turner, Williams syndrome: 2–12%
- First-degree relative with coeliac disease: 2–20%

% prevalence listed for each condition.[3,17]

American population carry either one or both of these genes.[47] However, HLA-typing is useful to exclude coeliac disease because of its very high negative predictive value, for example in children who have already started a GFD without prior diagnostic tests. HLA-typing is also useful in selecting individuals at risk of coeliac disease that need to undergo serological coeliac disease screening. Parents of affected children support HLA-typing of their other children to assess the risk of the disease.[48]

Histology

The characteristic histological alterations of the small bowel mucosa in coeliac disease are partial to total villous atrophy with crypt hyperplasia and IEL infiltration.[49,50] These alterations are rated according to the Marsh–Oberhuber classification depending on the severity of the lesion: ranging from type 0 (normal) to 4, wherein type 4 describes hypoplastic lesions.[49,50] When interpreting the histological alterations one should take the patient's serology, HLA-typing and clinical manifestations into account. A Marsh–Oberhuber classification type 3 (a, b or c), or type 2 if accompanied by specific coeliac disease antibodies, support the diagnosis of coeliac disease. The severity of the clinical symptoms does not correlate with the severity of the histological alterations. Patients with Marsh–Oberhuber type 3c can be asymptomatic.[4,14,17,51]

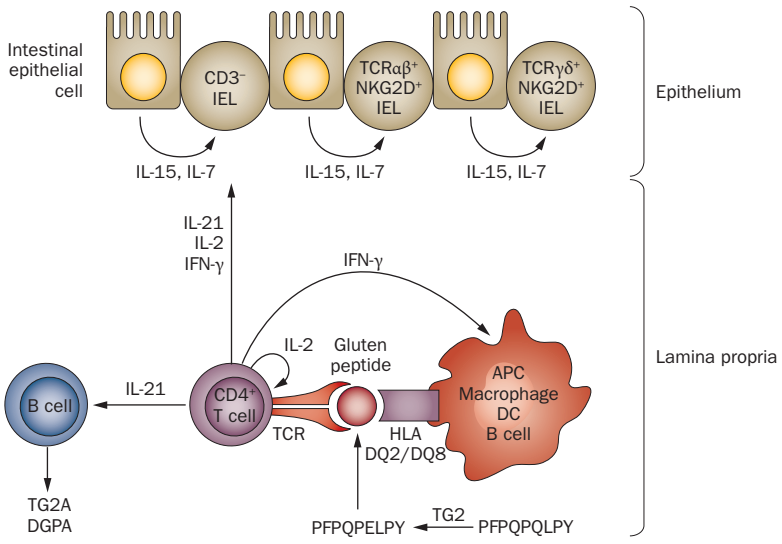

Figure 2 | Schematic representation of the immune response to gluten peptides in the small bowel mucosa of patients with coeliac disease. Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; DGPA, anti-deamidated gliadin peptide antibody; IEL, intraepithelial lymphocyte; NKG2D, NKG2-D type II integral membrane protein; TCR, T-cell receptor; TG2, transglutaminase 2; TG2A, anti-transglutaminase type 2 antibody.

Up until the past few years, the histological examination of small bowel biopsies was the gold standard for the diagnosis of coeliac disease. However, in 2012, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) introduced an exception for a specific group of children (Figure 3).[2] Small bowel biopsies can be omitted in children with clear gastrointestinal symptoms, high titres of TG2A (>10 upper limit of normal), positive EMA and HLA-DQ2 and/or HLA-DQ8. In all other cases, small bowel biopsies are still mandatory for diagnosis (Figure 4). The results of the ongoing prospective study ProCeDe investigating the performance of the ESPGHAN guidelines will be important to further define situations in which coeliac disease might be diagnosed without biopsies.[52]

Management

Coeliac disease can be successfully treated with a GFD, which restores small bowel histology and improves clinical complaints in the majority of patients.[46] Adhering to a GFD might seem simple, but the abundance of gluten-containing food in the Western diet can be challenging and treatment can considerably affect the child's quality of life.[53,54] Once diagnosis is confirmed, the child should be referred to a paediatric dietitian for in-depth information about the necessary dietary treatment. The GFD can have negative nutritional consequences. For instance, it has been reported that Italian adolescents with coeliac disease consumed an unbalanced diet rich in fat and protein, poor in carbohydrate and deficient in calcium, iron and fibre as a result of a GFD.[55] Gluten-containing cereals such as wheat, barley and rye are important sources of dietary iron, fibre, calcium, folate and vitamin B_{12}, and treatment with a GFD can lead to micronutrient deficiencies.[56,57] Gluten-free buckwheat or quinoa are naturally rich in group B vitamins,[58] but commercially available gluten-free products frequently do not contain the same amount of micronutrients as the often enriched wheat flour products that they aim to replace.[59] Noncontaminated oats are generally well tolerated by the majority of children with coeliac disease. However, a randomized double-blind study published in 2014 showed that oats prevent normalization of the intestinal mucosa immune status in a substantial fraction of paediatric patients with coeliac disease.[60]

The usual care for children with coeliac disease consists of hospital visits to monitor the patient's response to the diet. Subsequent follow-up is dedicated to assess the child's dietary adherence, well-being and adequacy of growth. Determination of coeliac-disease-specific antibodies in the serum should be done periodically to monitor regression and remission; their levels usually returning to normal within 9–12 months after dietary intervention.[61] Testing for anaemia, iron status and calcium, folic acid, vitamins D and B12 levels at diagnosis and at the follow-up visits of patients undergoing treatment is common practice. However, evidence is weak for the efficacy and adequacy of this practice as there is limited information on the incidence of nutritional deficiences in patients treated with coeliac disease.

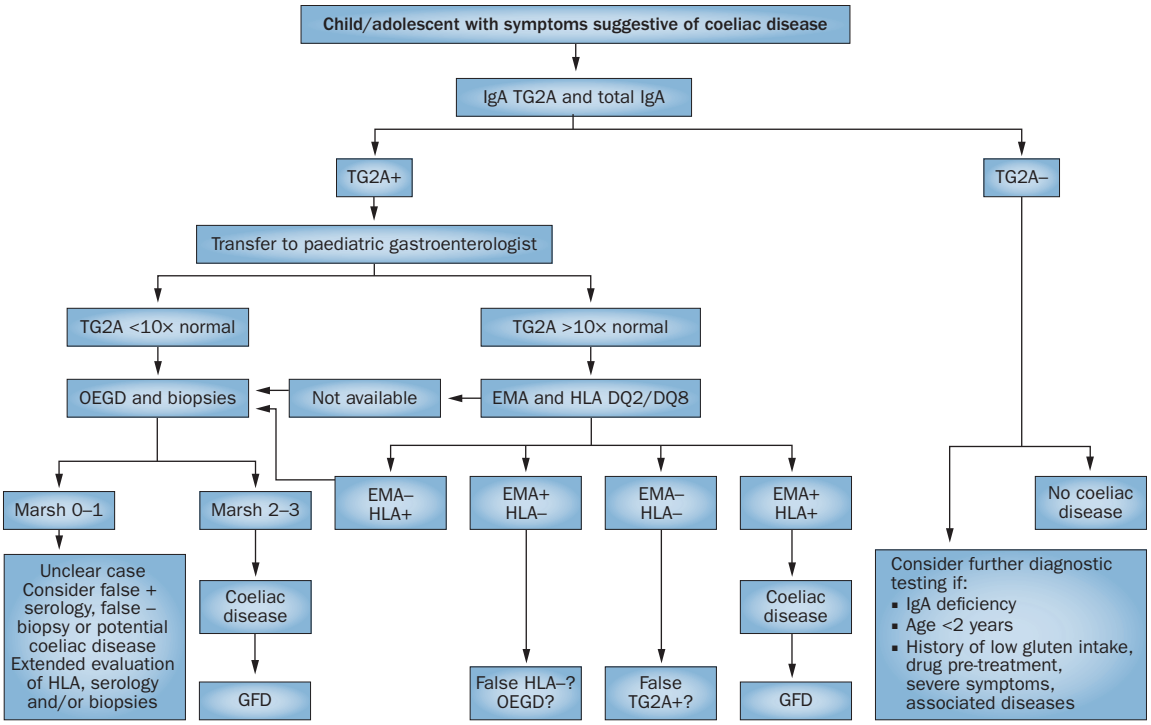

Figure 3 | ESPGHAN algorithm for the diagnosis of coeliac disease in children and adolescents with symptoms. Abbreviations: +, positive; –, negative; EMA; anti-endomysium antibody; ESPGHAN, European Society of Paediatric Gastroenterology, Hepatology and Nutrition; GFD, gluten-free diet; OEGD, oesophagogastroduodenoscopy; TG2A, anti-transglutaminase type 2 antibody. Permission obtained from Wolters Kluwer Health, Inc. © Husby, S. *et al. J. Pediatr. Gastroenterol. Nutr.* **54**, 136–160 (2012).

The evidence-based British and Dutch guidelines recommend annual visits whereas other evidence-based guidelines, such as the ones from the NIH, ESPGHAN, and the North American Society for Paediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) do not provide guidance on the matter.[2,62–65]

Novel therapies

Knowledge of the molecular mechanisms underlying coeliac disease offers opportunities to develop alternative treatments to the GFD.[66] The use of enzymes as oral supplements to enhance gluten degradation has been extensively studied and could help reduce gluten exposure.[67,68] Alternatively, the generation of blockers to prevent gluten peptide binding to HLA-DQ2 has been explored.[69,70] Similarly, blockade of TG2 would prevent gluten modification and the development of a full-blown T-cell response to gluten.[71] In addition, gluten peptide vaccination to re-introduce gluten tolerance has been proposed,[72] whereas other studies aim to improve barrier function in the small intestine to prevent the entry of gluten peptides into the lamina propria.[73] So far, none of these approaches has proven capable of replacing the GFD.

Prevention

Until now, primary prevention of coeliac disease is not possible. Previous retrospective studies suggested a 'window of opportunity' for primary prevention by introducing gluten between 4–6 months of age.[74,75] ESPGHAN recommends that gluten should not be introduced before 17 weeks and not later than 26 weeks of age, preferably concurrent with the period of breastfeeding.[76,77] However, the results from prospective studies and a systemic review of the literature establish that the timing of gluten introduction and the duration or maintenance of breastfeeding do not influence the development of coeliac disease into childhood.[17,51,78–80] These studies also show that the only identified risk factors for coeliac disease later in life are female gender, the HLA genotype and being born in Sweden.[51,81]

Secondary prevention of coeliac disease is possible through early diagnosis and treatment. As coeliac disease is severely underdiagnosed, the only way to achieve large-scale secondary prevention is by mass screening.[14,82,83] However, health improvement secondary to the treatment of the disease among minimally symptomatic or asymptomatic patients identified by mass screening makes this subject controversial.[83,84] Nevertheless, results of prospective studies indicate that important health problems, such as reduced fetal growth and birth weight, delayed growth in height and weight in children, and reduced bone mineral density in both children and adults, can be prevented by mass screening.[78,85,86] These results might reopen the debate on the topic in the future.[87] Adherence to a strict GFD can be considered as tertiary prevention for the disease as it reduces the long-term complications of coeliac disease such as chronic anaemia, infertility, autoimmune disorders, malignancy and osteoporosis.[46]

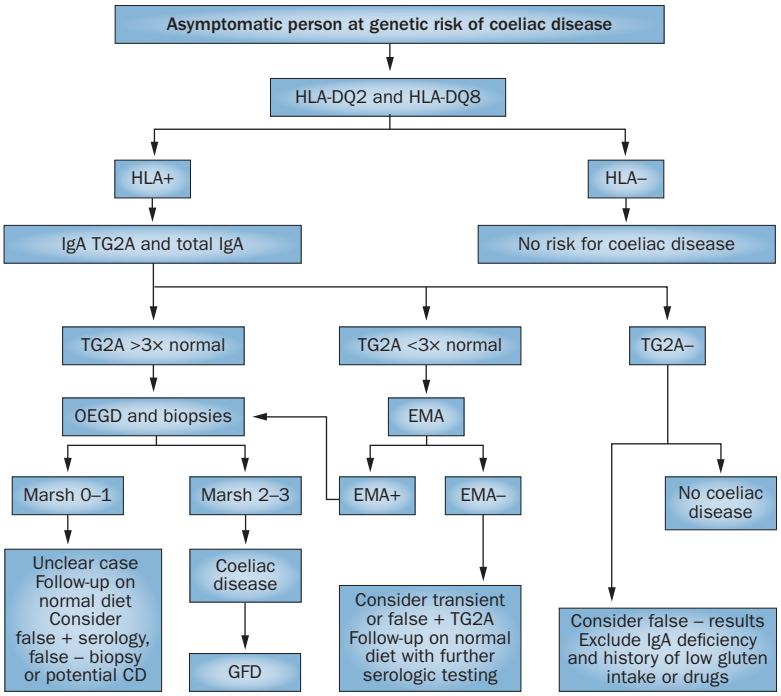

Figure 4 | ESPGHAN algorithm for the diagnosis of coeliac disease in asymptomatic children and adolescents with a genetic risk of coeliac disease. Abbreviations: +, positive; –, negative; EMA; anti-endomysium antibody; ESPGHAN, European Society of Paediatric Gastroenterology, Hepatology and Nutrition; GFD, gluten-free diet; OEGD, oesophagogastroduodenoscopy; TG2A, anti-transglutaminase type 2 antibody. Permission obtained from Wolters Kluwer Health, Inc. © Husby, S. *et al. J. Pediatr. Gastroenterol. Nutr.* **54**, 136–160 (2012).

Wheat allergy

Wheat allergy is defined as an IgE-mediated reaction to wheat proteins.[88,89] Depending on the route of exposure, wheat allergy is classified into: classic food allergy affecting the skin, gastrointestinal, or respiratory tract; wheat-dependent exercise-induced anaphylaxis (WDEIA); baker's asthma induced by inhalation of raw wheat flour, causing rhinitis and respiratory distress; and contact urticaria.[88] Children can present with immediate symptoms ranging from urticaria to angioedema and shock. Symptoms of delayed hypersensitivity appear ~24 h after wheat ingestion and can include gastrointestinal symptoms, pruritus or an eczematous rash. WDEIA is an allergic reaction induced by the combination of wheat after physical activity. Sometimes an additional trigger is needed, for example cold stimulation.[88,90]

Wheat allergy is one of the most common food allergies in children, and usually begins in early childhood and is less commonly seen in adolescents and adults.[88,91,92] A systemic review published in 2014 reported wheat allergy, after cow's milk allergy, as the second most frequent self-reported food allergy with an overall pooled estimate for lifetime prevalence of 3.6% (95% CI 3.0–4.2) but only with 0.1% point-prevalence [95% CI 0.01–0.2] of objectively verified wheat allergy by food challenge,[92] as had been shown before for children (>1% versus <1%).[93]

Similar to other forms of allergy, a positive family history of atopy is a predictor of subsequent wheat allergy in children.[94] According to the prospective DAISY cohort, the risk of developing wheat allergy increased fourfold when infants were introduced to gluten between 4 and 6 months of age.[95] In this cohort, breastfeeding did not protect against wheat allergy.[95] The use of antibiotics early in life has been associated with an increased risk of atopic disease, including food allergy.[96]

Pathogenesis

In patients with wheat allergy, ingestion of wheat elicits either an immediate IgE-mediated reaction or a delayed antibody-independent T-cell mediated reaction to a spectrum of different wheat protein fractions.[88] In IgE-mediated hypersensitivities, wheat-specific IgE is produced, which is then bound to high-affinity IgE receptors on mast cells or basophils. Re-exposure to wheat causes cell-bound IgE and wheat allergen to crosslink, causing degranulation of, among others, histamine, prostaglandins and leukotrienes.[97] In WDEIA, the most important allergens are the ω–5 gliadins and high-molecular-weight glutenin subunits.[88,98] In classic wheat allergy in children with atopic dermatitis, sensitization to α-amylase trypsin inhibitors is well-documented.[88,99] Both α-amylase trypsin inhibitors and gliadins are important allergens in baker's asthma as well.[98] Children with wheat allergy can usually tolerate other prolamine-containing cereals such as rye or barley, and their wheat-free diet is less restrictive than the GFD for coeliac disease.[100] However, the limits set for patients with coeliac disease can safely be applied to patients with wheat allergy.[101]

Diagnosis

The double-blind, placebo-controlled food challenge (DBPCFC) is considered as the gold standard for diagnosis of food allergies. This food challenge is, however, time-consuming, difficult for the patient and health-care professional, and associated with a risk of anaphylaxis. Other frequently used methods are wheat-specific IgE testing (RAST), skin-prick tests and patch tests, but they are not an alternative for food challenges. Both RAST and skin-prick tests indicate the presence of IgE sensitization to wheat,[102] with reported sensitivities of 83% and 73%, respectively.[103] However, specificities are low (43% and 73%, respectively) in part because wheat-specific IgE is common among children with atopy without true wheat allergy.[103] In addition, the RAST test detects only part of the wheat protein, whereas a large number of different wheat proteins are responsible for the allergic reaction.[98] Measurement of IgE sensitization to wheat is not suitable for diagnosing delayed non-IgE-mediated responses to wheat. In this case, a patch test can be helpful. The limited available evidence reported good specificity (89%) but weak sensitivity (29%).[103]

Management

The treatment of wheat allergy other than WDEIA is based on the avoidance of wheat products. The main prevention strategy of WDEIA is exercise avoidance for ~4 h after wheat ingestion. Moreover, wheat intake is discouraged 4 h after aspirin or other cyclooxygenase-1 inhibitor intake as it might enhance WDEIA symptoms, for reasons

not completely elucidated.[104] All patients should consult a dietitian for training in reading food labels. Patients should also be educated in using epinephrine autoinjectors if the onset of the allergic response is immediate and unpredictable.[88] In comparison with other food allergies, the prognosis for wheat allergy is relatively good: the majority of children have outgrown their allergy by age 12 years.[105]

Noncoeliac gluten sensitivity

NCGS is a clinical condition in which intestinal and extraintestinal symptoms are triggered by gluten ingestion, in the absence of coeliac disease and wheat allergy. The symptoms usually occur soon after gluten ingestion, improve or disappear within hours or a few days after gluten withdrawal, and relapse following its reintroduction.[106] NCGS intestinal symptoms are nonspecific and IBS-like, presenting with abdominal pain, bloating and diarrhoea. Extraintestinal symptoms include fatigue, headache, joint and/or muscle pain, weight loss, anaemia, dermatitis and behavioural disturbances.[106–108] Children with NCGS most frequently have abdominal pain and diarrhoea and, less frequently, systemic manifestations.[109] An association between NCGS and neuropsychiatric disorders, such as schizophrenia and autism spectrum disorders, has been suggested.[110,111]

Pathogenesis

The pathogenesis of NCGS is unknown. Thus far, there are no randomized controlled trials examining NCGS in children and, to the best of our knowledge, only one clinical study concerning NCGS in children has been published.[109] In the small bowel mucosa of adult patients with NCGS the literature is in agreement that only minor histological alterations are found, compatible with Marsh–Oberhuber 0–1 classifications.[107,112,113] Some studies have reported normal intestinal permeability, whereas others reported increased permeability in a subgroup of HLA-DQ2/DQ8-positive adult patients.[1,107,114,115] Gene expression analyses showed increased expression of Toll-like receptor 2 (TLR2) and reduced expression of the regulatory-T-cell marker FOXP3 in patients with NCGS in comparison to patients with coeliac disease, suggesting a pathogenic role for innate immunity. Contrary to coeliac disease, most studies show that levels of adaptive immunity markers are not increased in NCGS.[1] Other grain proteins different from gluten might also be responsible for the symptoms in NCGS. For example, the α-amylase trypsin inhibitors, which are groups of low-molecular-weight proteins in wheat and related cereals, are strong activators of innate immune responses and it has been hypothesized that they might have a role in NCGS.[116] However, to date there is no clinical data to support this hypothesis.

Diagnosis

NCGS is a diagnosis of exclusion after ruling out other gluten-related disorders, especially coeliac disease and wheat allergy. No biological markers are available for NCGS. The only antibodies observed in a retrospective study of adults are IgG and IgA anti-gliadin antibodies, which occur in 56% and 8% of the patients, respectively, in comparison to 80% and 75%, respectively, in the coeliac disease population.[117] However, anti-gliadin antibodies are also frequently present in the general population. Half of the patients with NCGS were HLA-DQ2 or HLA-DQ8 positive, a prevalence only slightly higher than in the general population (40%).[106,108] A DBPCFC has been suggested to confirm NCGS diagnosis. This approach is quite a complicated procedure in practice, given the difficulty in preparing the intervention products, the need for highly trained personnel and high costs.[118] An alternative is the open food challenge, but this might render false-positive results.[119] Confirming the diagnosis of NCGS is important to avoid unnecessary dietary restrictions and the possibility of missing coeliac disease.[120] However, for research concerning NCGS the diagnosis should be confirmed with a DBPCFC.

Management

In general, NCGS, as with coeliac disease and wheat allergy, is treated with a GFD but because of the lack of knowledge about its gluten (dose)-related character and the permanent or transient nature of the condition, periodic reintroduction of gluten into the diet could be advised.[106,117] The main effect of the GFD in patients with NCGS has been ascribed to an improvement in the perception of their general well-being.[121] In a double-blind randomized placebo-controlled trial in 34 adults with IBS without coeliac disease, symptom control was markedly better in patients on a GFD than for patients on a gluten-containing diet.[107] In another randomized controlled trial in 45 gluten-ingesting adults with IBS without coeliac disease, a small positive effect of a GFD on stool frequency was shown, but not on stool consistency.[115] Subsequently, in a double-blind crossover trial of 37 individuals with NCGS and IBS but not coeliac disease, it was shown that symptoms clearly reduced on a low FODMAP (fermentable oligosaccharide, disaccharide, monosaccharide and polyol) diet, challenging the assumption that gluten is causing NGCS.[122] In a Cochrane review including two small randomized controlled trials, no evidence of efficacy of gluten exclusion in neuropsychiatric disorders was found.[87,123]

Conclusions

The incidence of gluten-related disorders, including coeliac disease, is increasing. Differentiating the gluten-related disorders solely based on clinical manifestations is impossible. Although the available diagnostic tests for coeliac disease have improved greatly in the past few years, a large number of patients remain undiagnosed. This finding might reopen the debate on mass screening for coeliac disease. For the growing number of patients with gluten-related symptoms without coeliac disease or wheat allergy, no objective diagnostic tools are available. Together with a group of disease-free individuals who choose gluten free food because they think these products are healthier in general, they have contributed to the growing popularity of gluten-free food. Objective diagnostic tools are warranted for further evaluation of the incidence and prevalence of these different gluten-related disorders in children and in adults.

1. Meijer, C. R., Shamir, R. & Mearin, M. L. Coeliac disease and gluten sensitivity. *J. Pediatr. Gastroenterol. Nutr.* **60**, 429–432 (2015).
2. Husby, S. *et al.* European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J. Pediatr. Gastroenterol. Nutr.* **54**, 136–160 (2012).
3. Myleus, A. *et al.* Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. *J. Pediatr. Gastroenterol. Nutr.* **49**, 170–176 (2009).
4. Catassi, C., Gatti, S. & Fasano, A. The new epidemiology of celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **59** (Suppl. 1), S7–S9 (2014).
5. Gandolfi, L. *et al.* Prevalence of celiac disease among blood donors in Brazil. *Am. J. Gastroenterol.* **95**, 689–692 (2000).
6. Gomez, J. C. *et al.* Prevalence of celiac disease in Argentina: screening of an adult population in the La Plata area. *Am. J. Gastroenterol.* **96**, 2700–2704 (2001).
7. Barada, K., Bitar, A., Mokadem, M. A., Hashash, J. G. & Green, P. Celiac disease in Middle Eastern and North African countries: a new burden? *World J. Gastroenterol.* **16**, 1449–1457 (2010).
8. Masjedizadeh, R. *et al.* Celiac disease in South-West of Iran. *World J. Gastroenterol.* **12**, 4416–4419 (2006).
9. Yuan, J. *et al.* The tip of the "celiac iceberg" in China: a systematic review and meta-analysis. *PLoS ONE* **8**, e81151 (2013).
10. Byass, P., Kahn, K. & Ivarsson, A. The global burden of childhood coeliac disease: a neglected component of diarrhoeal mortality? *PLoS ONE* **6**, e22774 (2011).
11. Fasano, A. *et al.* Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition consensus report on celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **47**, 214–219 (2008).
12. Rubio-Tapia, A. *et al.* Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology* **137**, 88–93 (2009).
13. Catassi, C. *et al.* Detection of Celiac disease in primary care: a multicenter case-finding study in North America. *Am. J. Gastroenterol.* **102**, 1454–1460 (2007).
14. Csizmadia, C. G., Mearin, M. L., von Blomberg, B. M., Brand, R. & Verloove-Vanhorick, S. P. An iceberg of childhood coeliac disease in the Netherlands. *Lancet* **353**, 813–814 (1999).
15. Sandstrom, O. *et al.* Transglutaminase IgA antibodies in a celiac disease mass screening and the role of HLA-DQ genotyping and endomysial antibodies in sequential testing. *J. Pediatr. Gastroenterol. Nutr.* **57**, 472–476 (2013).
16. Steens, R. F. *et al.* A national prospective study on childhood celiac disease in the Netherlands 1993–2000: an increasing recognition and a changing clinical picture. *J. Pediatr.* **147**, 239–243 (2005).
17. Vriezinga, S. L. *et al.* Randomized feeding intervention in infants at high risk for celiac disease. *N. Engl. J. Med.* **371**, 1304–1315 (2014).
18. Lundin, K. E. *et al.* Gliadin-specific, HLA-DQ(α 1*0501, β 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J. Exp. Med.* **178**, 187–196 (1993).
19. Anderson, R. P., Degano, P., Godkin, A. J., Jewell, D. P. & Hill, A. V. *In vivo* antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. *Nat. Med.* **6**, 337–342 (2000).
20. Arentz-Hansen, H. *et al.* The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J. Exp. Med.* **191**, 603–612 (2000).
21. Shan, L. *et al.* Structural basis for gluten intolerance in celiac sprue. *Science* **297**, 2275–2279 (2002).
22. Sjostrom, H. *et al.* Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand. J. Immunol.* **48**, 111–115 (1998).
23. Tye-Din, J. A. *et al.* Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. *Sci. Transl. Med.* **2**, 41ra51 (2010).
24. Vader, L. W. *et al.* Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology* **125**, 1105–1113 (2003).
25. Vader, W. *et al.* The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology* **122**, 1729–1737 (2002).
26. van de Wal, Y. *et al.* Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc. Natl. Acad. Sci. USA* **95**, 10050–10054 (1998).
27. van de Wal, Y. *et al.* Glutenin is involved in the gluten-driven mucosal T cell response. *Eur. J. Immunol.* **29**, 3133–3139 (1999).
28. Molberg, O. *et al.* Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat. Med.* **4**, 713–717 (1998).
29. Vader, L. W. *et al.* Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J. Exp. Med.* **195**, 643–649 (2002).
30. van de Wal, Y. *et al.* Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. *J. Immunol.* **161**, 1585–1588 (1998).
31. Mearin, M. L. *et al.* HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease. *Gut* **24**, 532–537 (1983).
32. Vader, W. *et al.* The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T cell responses. *Proc. Natl. Acad. Sci. USA* **100**, 12390–12395 (2003).
33. Vermeulen, B. A. *et al.* Phenotypic variance in childhood coeliac disease and the HLA-DQ/DR dose effect. *Scand. J. Gastroenterol.* **44**, 40–45 (2009).
34. Tjon, J. M., van, B. J. & Koning, F. Celiac disease: how complicated can it get? *Immunogenetics* **62**, 641–651 (2010).
35. van, Bergen, J., Mulder, C. J., Mearin, M. L. & Koning, F. Local communication among mucosal immune cells in patients with celiac disease. *Gastroenterology* **148**, 1187–1194 (2015).
36. Abadie, V., Sollid, L. M., Barreiro, L. B. & Jabri, B. Integration of genetic and immunological insights into a model of celiac disease pathogenesis. *Annu. Rev. Immunol.* **29**, 493–525 (2011).
37. Jarvinen, T. T. *et al.* Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease. *Scand. J. Gastroenterol.* **39**, 428–433 (2004).
38. Meresse, B. *et al.* Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity* **21**, 357–366 (2004).
39. Hue, S. *et al.* A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* **21**, 367–377 (2004).
40. Kutlu, T. *et al.* Numbers of T cell receptor (TCR) αβ+ but not TcR γδ+ intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet. *Gut* **34**, 208–214 (1993).
41. Schmitz, F. *et al.* Identification of a potential physiological precursor of aberrant cells in refractory coeliac disease type II. *Gut* **62**, 509–519 (2013).
42. Ludvigsson, J. F. & Green, P. H. The missing environmental factor in celiac disease. *N. Engl. J. Med.* **371**, 1341–1343 (2014).
43. Olivares, M. *et al.* The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. *Gut* **64**, 406–417 (2015).
44. Guandalini, S. & Assiri, A. Celiac disease: a review. *JAMA Pediatr.* **168**, 272–278 (2014).
45. Giersiepen, K. *et al.* Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. *J. Pediatr. Gastroenterol. Nutr.* **54**, 229–241 (2012).
46. Green, P. H. & Jabri, B. Coeliac disease. *Lancet* **362**, 383–391 (2003).
47. Hadithi, M. *et al.* Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. *Ann. Intern. Med.* **147**, 294–302 (2007).
48. Wessels, M. M. *et al.* Impact on parents of HLA-DQ2/DQ8 genotyping in healthy children from coeliac families. *Eur. J. Hum. Genet.* **23**, 405–408 (2014).
49. Marsh, M. N. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* **102**, 330–354 (1992).
50. Oberhuber, G., Granditsch, G. & Vogelsang, H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur. J. Gastroenterol. Hepatol.* **11**, 1185–1194 (1999).
51. Aronsson, C. A. *et al.* Age at gluten introduction and risk of celiac disease. *Pediatrics* **135**, 239–245 (2015).
52. Werkstetter, K. *ProCeDE: Prospective Celiac Disease Diagnostic Evaluation* [online], http://procede2011.jimdo.com/ (2015).
53. Kolsteren, M. M., Koopman, H. M., Schalekamp, G. & Mearin, M. L. Health-related quality of life in children with celiac disease. *J. Pediatr.* **138**, 593–595 (2001).
54. van Doorn, R. K., Winkler, L. M., Zwinderman, K. H., Mearin, M. L. & Koopman, H. M. CDDUX: a disease-specific health-related quality-of-life questionnaire for children with celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **47**, 147–152 (2008).
55. Mariani, P. *et al.* The gluten-free diet: a nutritional risk factor for adolescents with celiac disease? *J. Pediatr. Gastroenterol. Nutr.* **27**, 519–523 (1998).
56. Hopman, E. G., le Cessie, S., von Blomberg, B. M. & Mearin, M. L. Nutritional management of the gluten-free diet in young people with celiac disease in The Netherlands. *J. Pediatr. Gastroenterol. Nutr.* **43**, 102–108 (2006).
57. Ohlund, K., Olsson, C., Hernell, O. & Ohlund, I. Dietary shortcomings in children on a gluten-free diet. *J. Hum. Nutr. Diet.* **23**, 294–300 (2010).
58. Alvarez-Jubete, L., Arendt, E. K. & Gallagher, E. Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. *Int. J. Food Sci. Nutr.* **60** (Suppl. 4), 240–257 (2009).

59. do Nascimento, A. B., Fiates, G. M., Dos, A. A. & Teixeira, E. Analysis of ingredient lists of commercially available gluten-free and gluten-containing food products using the text mining technique. *Int. J. Food Sci. Nutr.* **64**, 217–222 (2013).
60. Sjoberg, V. *et al.* Noncontaminated dietary oats may hamper normalization of the intestinal immune status in childhood celiac disease. *Clin. Transl. Gastroenterol.* **5**, e58 (2014).
61. Hogen Esch, C. E. *et al.* Specific celiac disease antibodies in children on a gluten-free diet. *Pediatrics* **128**, 547–552 (2011).
62. James, S. P. National Institutes of Health Consensus Development Conference Statement on Celiac Disease, June 28–30, 2004. *Gastroenterology* **128** (Suppl. 1), S1–S9 (2005).
63. Nederlandse Vereniging van Maag-Darm-Leverartsen. Richtlijn Coeliakie en Dermatitis Herpetiformis Richtlijn Coeliakie en Dermatitis Herpetiformis. *Haarlem: Nederlandse Vereniging voor Maag-Darm-Leverartsen* [online], http://www.mdl.nl/uploads/240/442/richtlijn_Coeliakie_definitief.pdf (2008).
64. Hill, I. D. *et al.* Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J. Pediatr. Gastroenterol. Nutr.* **40**, 1–19 (2005).
65. Murch, S. *et al.* Joint BSPGHAN and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children. *Arch. Dis. Child* **98**, 806–811 (2013).
66. Freeman, H. J. Non-dietary forms of treatment for adult celiac disease. *World J. Gastrointest. Pharmacol. Ther.* **4**, 108–112 (2013).
67. Mitea, C. *et al.* Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. *Gut* **57**, 25–32 (2008).
68. Siegel, M. *et al.* Safety, tolerability, and activity of ALV003: results from two phase 1 single, escalating-dose clinical trials. *Dig. Dis. Sci.* **57**, 440–450 (2012).
69. Kapoerchan, V. V. *et al.* Design, synthesis and evaluation of high-affinity binders for the celiac disease associated HLA-DQ2 molecule. *Mol. Immunol.* **47**, 1091–1097 (2010).
70. Xia, J. *et al.* Cyclic and dimeric gluten peptide analogues inhibiting DQ2-mediated antigen presentation in celiac disease. *Bioorg. Med. Chem.* **15**, 6565–6573 (2007).
71. Klock, C., Herrera, Z., Albertelli, M. & Khosla, C. Discovery of potent and specific dihydroisoxazole inhibitors of human transglutaminase 2. *J. Med. Chem.* **57**, 9042–9064 (2014).
72. Keech, C. L., Dromey, J. A., Chen, Z., Anderson, R. P. & McCluskey, J. Immune tolerance induced by peptide immunotherapy in an HLA Dq2-dependent mouse model of gluten immunity [abstract 355]. *Gastroenterology* **136**, A57 (2009).
73. Kelly, C. P. *et al.* Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study. *Aliment. Pharmacol. Ther.* **37**, 252–262 (2013).
74. Ivarsson, A. *et al.* Epidemic of coeliac disease in Swedish children. *Acta Paediatr.* **89**, 165–171 (2000).
75. Norris, J. M. *et al.* Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. *JAMA* **293**, 2343–2351 (2005).
76. Agostoni, C. *et al.* Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J. Pediatr. Gastroenterol. Nutr.* **46**, 99–110 (2008).
77. Akobeng, A. K., Ramanan, A. V., Buchan, I. & Heller, R. F. Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies. *Arch. Dis. Child* **91**, 39–43 (2006).
78. Jansen, M. A. *et al.* Infant feeding and anti-tissue transglutaminase antibody concentrations in the Generation R Study. *Am. J. Clin. Nutr.* **100**, 1095–1101 (2014).
79. Lionetti, E. *et al.* Introduction of gluten, HLA status, and the risk of celiac disease in children. *N. Engl. J. Med.* **371**, 1295–1303 (2014).
80. Szajewska, H. *et al.* Systematic review with meta-analysis: early infant feeding and coeliac disease—update 2015. *Aliment. Pharmacol. Ther.* **41**, 1038–1054 (2015).
81. Ivarsson, A. *et al.* Prevalence of childhood celiac disease and changes in infant feeding. *Pediatrics* **131**, e687–e694 (2013).
82. Rosen, A. *et al.* Usefulness of symptoms to screen for celiac disease. *Pediatrics* **133**, 211–218 (2014).
83. Mearin, M. L., Ivarsson, A. & Dickey, W. Coeliac disease: is it time for mass screening? *Best. Pract. Res. Clin. Gastroenterol.* **19**, 441–452 (2005).
84. van Koppen, E. J. *et al.* Long-term health and quality-of-life consequences of mass screening for childhood celiac disease: a 10-year follow-up study. *Pediatrics* **123**, e582–e588 (2009).
85. Kiefte-de Jong, J. C. *et al.* Levels of antibodies against tissue transglutaminase during pregnancy are associated with reduced fetal weight and birth weight. *Gastroenterology* **144**, 726–735 (2013).
86. Kurppa, K. *et al.* Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease. *Gastroenterology* **147**, 610–617 (2014).
87. Catassi, C. & Fasano, A. Coeliac disease. The debate on coeliac disease screening—are we there yet? *Nat. Rev. Gastroenterol. Hepatol.* **11**, 457–458 (2014).
88. Inomata, N. Wheat allergy. *Curr. Opin. Allergy Clin. Immunol.* **9**, 238–243 (2009).
89. Johansson, S. G. *et al.* Revised nomenclature for allergy for global use: report of the nomenclature review committee of the World Allergy Organization, October 2003. *J. Allergy Clin. Immunol.* **113**, 832–836 (2004).
90. Benhamou, A. H., Vanini, G., Lantin, J. P. & Eigenmann, P. A. Antihistamine and sodium cromoglycate medication for food cold water exercise-induced anaphylaxis. *Allergy* **62**, 1471–1472 (2007).
91. Mulder, C. J., van Wanrooij, R. L., Bakker, S. F., Wierdsma, N. & Bouma, G. Gluten-free diet in gluten-related disorders. *Dig. Dis.* **31**, 57–62 (2013).
92. Nwaru, B. I. *et al.* Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* **69**, 992–1007 (2014).
93. Zuidmeer, L. *et al.* The prevalence of plant food allergies: a systematic review. *J. Allergy Clin. Immunol.* **121**, 1210–1218 (2008).
94. Zeiger, R. S. & Heller, S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. *J. Allergy Clin. Immunol.* **95**, 1179–1190 (1995).
95. Poole, J. A. *et al.* Timing of initial exposure to cereal grains and the risk of wheat allergy. *Pediatrics* **117**, 2175–2182 (2006).
96. Droste, J. H. *et al.* Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? *Clin. Exp. Allergy* **30**, 1547–1553 (2000).
97. Husain, Z. & Schwartz, R. A. Food allergy update: more than a peanut of a problem. *Int. J. Dermatol.* **52**, 286–294 (2013).
98. Makela, M. J. *et al.* Wheat allergy in children—new tools for diagnostics. *Clin. Exp. Allergy* **44**, 1420–1430 (2014).
99. Leonard, M. M. & Vasagar, B. US perspective on gluten-related diseases. *Clin. Exp. Gastroenterol.* **7**, 25–37 (2014).
100. Jones, S. M., Magnolfi, C. F., Cooke, S. K. & Sampson, H. A. Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. *J. Allergy Clin. Immunol.* **96**, 341–351 (1995).
101. Hischenhuber, C. *et al.* Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. *Aliment. Pharmacol. Ther.* **23**, 559–575 (2006).
102. Yang, H., Xiao, Y. Z., Luo, X. Y., Tan, Q. & Wang, H. Diagnostic accuracy of atopy patch tests for food allergy in children with atopic dermatitis aged less than two years. *Allergol. Immunopathol. (Madr.)* **42**, 22–28 (2014).
103. Soares-Weiser, K. *et al.* The diagnosis of food allergy: a systematic review and meta-analysis. *Allergy* **69**, 76–86 (2014).
104. Matsukura, S. *et al.* Two cases of wheat-dependent anaphylaxis induced by aspirin administration but not by exercise. *Clin. Exp. Dermatol.* **35**, 233–237 (2010).
105. Keet, C. A. *et al.* The natural history of wheat allergy. *Ann. Allergy Asthma Immunol.* **102**, 410–415 (2009).
106. Sapone, A. *et al.* Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med.* **10**, 13 (2012).
107. Biesiekierski, J. R. *et al.* Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. *Am. J. Gastroenterol.* **106**, 508–514 (2011).
108. Catassi, C. *et al.* Non-Celiac Gluten sensitivity: the new frontier of gluten related disorders. *Nutrients.* **5**, 3839–3853 (2013).
109. Francavilla, R. *et al.* Clinical, serologic, and histologic features of gluten sensitivity in children. *J. Pediatr.* **164**, 463–467 (2014).
110. Batista, I. C. *et al.* Autism spectrum disorder and celiac disease: no evidence for a link. *Arq Neuropsiquiatr.* **70**, 28–33 (2012).
111. Whiteley, P. *et al.* The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. *Nutr. Neurosci.* **13**, 87–100 (2010).
112. Sapone, A. *et al.* Differential mucosal IL-17 expression in two gliadin-induced disorders: gluten sensitivity and the autoimmune enteropathy celiac disease. *Int. Arch. Allergy Immunol.* **152**, 75–80 (2010).
113. Volta, U., Bardella, M. T., Calabro, A., Troncone, R. & Corazza, G. R. An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity. *BMC Med.* **12**, 85 (2014).
114. Sapone, A. *et al.* Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med.* **9**, 23 (2011).

115. Vazquez-Roque, M. I. *et al.* A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function. *Gastroenterology* **144**, 903–911 (2013).
116. Junker, Y. *et al.* Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *J. Exp. Med.* **209**, 2395–2408 (2012).
117. Volta, U. & De, G. R. New understanding of gluten sensitivity. *Nat. Rev. Gastroenterol. Hepatol.* **9**, 295–299 (2012).
118. Lundin, K. E. & Alaedini, A. Non-celiac gluten sensitivity. *Gastrointest. Endosc. Clin. N. Am.* **22**, 723–734 (2012).
119. Ahrens, B., Niggemann, B., Wahn, U. & Beyer, K. Positive reactions to placebo in children undergoing double-blind, placebo-controlled food challenge. *Clin. Exp. Allergy* **44**, 572–578 (2014).
120. Biesiekierski, J. R., Newnham, E. D., Shepherd, S. J., Muir, J. G. & Gibson, P. R. Characterization of adults with a self-diagnosis of nonceliac gluten sensitivity. *Nutr. Clin. Pract.* **29**, 504–509 (2014).
121. Peters, S. L., Biesiekierski, J. R., Yelland, G. W., Muir, J. G. & Gibson, P. R. Randomised clinical trial: gluten may cause depression in subjects with non-coeliac gluten sensitivity—an exploratory clinical study. *Aliment. Pharmacol. Ther.* **39**, 1104–1112 (2014).
122. Biesiekierski, J. R. *et al.* No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology* **145**, 320–328 (2013).
123. Millward, C., Ferriter, M., Calver, S. & Connell-Jones, G. Gluten- and casein-free diets for autistic spectrum disorder. *Cochrane Database of Systematic* Reviews, Issue 1. Art. No.: CD003498 http://dx.doi.org/10.1002/14651858.CD003498.pub3 (2008).

Acknowledgements
We thank Dr D. Amado, visiting paediatrician at Leiden University Medical Centre, for editing the manuscript.

Author contributions
All authors contributed equally to all aspects of this manuscript.