Early diagnosis and management of celiac disease in childhood

Meijer, C.R.

Early diagnosis and management of celiac disease in childhood

Carolien Meijer

EARLY DIAGNOSIS AND MANAGEMENT OF CELIAC DISEASE IN CHILDHOOD

Carolien Renée Meijer

Layout and printing by Optima Grafische Communicatie (www.ogc.nl)

EARLY DIAGNOSIS AND MANAGEMENT OF CELIAC DISEASE IN CHILDHOOD

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof. dr. ir. H. Bijl,
volgens besluit van het college van promoties
te verdedigen op woensdag 25 januari 2023
klokke 10.00 uur

door

Carolien Renée Meijer
geboren te Gouda
in 1983

Promotoren:	Prof. dr. M.L. Mearin Manrique
	Prof. dr. E.H.H.M. Rings
Promotiecommissie:	Prof. dr. G. Bouma, Amsterdam Universitair Medisch Centrum
	Prof. dr. J.C. Escher, Sophia Kinderziekenhuis, Erasmus MC
	Prof. dr. F. Koning
	Prof. dr. T.W.J. Huizinga

Voor mijn nichtje Rosa

CONTENTS

1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated systemic disorder elicited by the ingestion of gluten containing cereals (among others wheat, rye and barley) from the normal diet in genetically susceptible individuals. CD is characterised by a variable combination of gluten-dependent clinical manifestations, CD specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy (1). CD may present with a large variety of nonspecific signs and symptoms. It is important to diagnose CD not only in children with obvious gastrointestinal symptoms but also in children with a less clear clinical picture (or without complaints) because the disease may have negative health consequences. However, one of the greatest challenges in childhood is to diagnose the disease timely and to manage it adequately.

Epidemiology

The genotypes HLA-DQ2 or HLA-DQ8 coded by chromosome 6, present in 40% of the general population, is necessary but not sufficient for CD to develop. The prevalence of CD has doubled in the past 50 years and currently affects about 1% of the world's population (2-7). Despite the increasing prevalence of CD, the rate of diagnosis has increased more slowly. The prevalence of undiagnosed CD remains substantial (5, 8-10). Because of the multitude of symptoms associated with CD, it is difficult to diagnose promptly and accurately. In addition, the clinical manifestation of CD has changed dramatically in the last decades from symptoms of malabsorption in childhood to milder manifestations or may even have no gastro-intestinal problems at all. Extra intestinal manifestations are more often presented at the time of diagnosis. Patients with atypical or nonspecific symptoms often report a delay in diagnosis of CD that may last for years (11) or even worse, CD remains unrecognized and, therefore, untreated (12-14). Untreated disease is associated with inflammation within the small intestine and villous atrophy leading to malabsorption, chronic anaemia, delayed puberty, neuropsychiatric disturbances, associated autoimmune disorders, infertility, small-for-date-births, osteoporosis and, rarely, malignancy and it can reduce the quality of life (QoL) (1, 15, 16).

Diagnosis

CD is characterized by the production of autoantibodies among others against transglutaminase type 2 (TG2A) and endomysium (EMA), during a period of gluten ingestion. Serological testing identifies most CD patients using CD-specific and -sensitive antibodies (17). Due to good accuracy of the serology-tests, ESPGHAN published in 2012 new guidelines for the diagnosis of CD in children and adolescents, including the novel so-called "non-biopsy approach" for selected cases (1).

However, TG2A measurement requires specialized laboratories, and the results are not immediately available. The call for point-of-care (POC) testing, defined as performing a diagnostic procedure outside the laboratory, has resulted in the commercial availability of several POC tests for TG2A. These tests obviate the need for purified or recombinant transglutaminase type 2 (TG2) or for serum separation because TG2 is also found in red blood cells (RBCs). Therefore, the patient's own TG2 can be used in TG2A detection by haemolysing a whole blood sample and liberating the self-TG2 from the RBCs. Tests can be performed at home or at the doctor's office and results become available within 10 minutes, which may save costs and prove to be more convenient for the patients. Several studies have investigated the accuracy of POC tests based on TG2A for CD screening, and sensitivities and specificities similar to those of determination of TG2A in serum were reported (70.1- 97% and 76-100% respectively) (18, 19).

Treatment

The only treatment available for CD is adhering to a gluten-free diet (GFD). Adherence to a GFD is widely accepted to be challenging; it can be influenced by many factors including, reduced QoL, symptoms on ingestion of gluten, knowledge of gluten free foods, understanding of food labels, cost and availability of gluten free foods including receiving GF foods on prescription, and membership of a celiac society. Adherence to a GFD ranges between 25-50% among children and adolescents with CD (20-22).

Treatment with a GFD restores small bowel histology, reduces the burden of morbidity and mortality associated with untreated CD and prevents complications on the long-term. Noncompliance can be intentional, but accidental gluten ingestion also happens because of contamination of non-toxic cereals such as oats or corn due to co-culture or spilling during food-processing either in factories or at home or during transport.

Follow up

General recommendations for follow up of CD patients differ substantially between countries and even regionally within countries applying the same healthcare system. Evidence on the frequency, who and what should be assessed during follow up is lacking. Clinical follow-up of children and adolescents with CD is necessary to assess the evolution of their symptoms as well as their growth and development and to monitor dietary compliance to the treatment with a GFD. Determination of TG2A, which usually disappear approximately 12 months after starting a GFD, is also performed during the follow up (23-26). The determinations are widely used during follow-up as a proxy for mucosal healing in CD children (27), but the results do not correlate well with diet compliance (22, 28, 29).

Despite the absence of a gold standard to assess dietary compliance, a dietary evaluation by a trained dietician is considered the best method, but this is time-consuming and requires expert personnel which is not always available. Short dietary questionnaires and TG2A determinations in serum fail to detect dietary transgressions in children and adolescents with CD, showing poor sensitivity to identify all patients who consume gluten (22, 30, 31). To assess the dietary compliance in children and adolescents with CD a dietary questionnaire has been developed and validated (22). Other methods, as measurement of gliadin immunogenic peptides (GIP) in urine and/or in faeces have been introduced to detect contaminating gluten into the GFD, but they are not used in the standard clinical care (32-34).

Traditional medical care for celiac patients consists of regular physician visits. The limited time allotted for outpatient follow-up also typically restricts comprehensive assessment of a patient's health-related quality of life (HRQoL) and dietary adherence (35). Self-management has shown beneficial effects on the healthcare of other chronic diseases. E-health can play an important role in supporting patients in their self-management, as internet and technology can reach users easily and rapidly, with a wide range of contents and attractive formats. E-health is defined as healthcare services and information delivered or enhanced electronically via the internet and related technologies. Work from our research group shows that online consultations for children and young adults with CD are cost saving, increase CD-specific QoL, and are satisfactory for the majority.

Prevention

Prevention is defined as any activity that reduces the burden of mortality or morbidity from disease, taking place at the primary (avoiding disease development), secondary (early detection and treatment) or tertiary level (avoiding complications by improved treatment) (36). The development of CD requires genetic susceptibility, present in 40% of the general population. However, only a minority of individuals genetically at risk of CD, 1%, develop the disease. So, environmental and/or lifestyle factors may play a causal role in the development of CD. Primary prevention strategies are not (yet) possible. Data from prospective studies of large cohorts evaluated the effect of the timing of gluten introduction on the risk of CD in at-risk children. Results have shown that neither the timing of gluten introduction nor the duration or maintenance of breastfeeding influence the risk of CD. Secondary prevention is possible through early diagnosis. Most international guidelines already recommend testing for CD in high-risk groups, such as first-degree relatives of CD patients (CD families) and patients with other autoimmune diseases. Case-finding and mass screening are still controversial because of the ethical implications. Active case finding refers to liberal diagnostic testing of patients with CD-

associated symptoms, while mass-screening refers to test the whole population for CD. However, since the clinical presentation of CD has changed dramatically in the last decades, patients with atypical or nonspecific symptoms often report a delay in diagnosis of CD that may last for years (11) or even worse, CD remains unrecognized and, therefore, untreated (12-14). Nowadays, regular follow up to ensure strict adherence to a GFD, is the only available, effective tertiary prevention option. Given that the GFD poses a major challenge and requires patient education, continuous motivation and follow-up, several trials are ongoing or underway to explore non-dietary treatment as possible options for tertiary prevention, but none of them have been tested in clinical trials yet.

OUTLINE OF THIS THESIS

The focus of this thesis is the improvement of diagnosis, early detection and treatment of CD in children. Increased knowledge, available guidelines and reliable diagnostics allow for timely diagnosis which can prevent complications and improve QoL, but the current healthcare approach is often unable to make the diagnosis in a timely manner. Moreover, despite timely diagnosis and effective therapy, there is a need to improve the follow up. **Chapter 2** describes the efficient implementation of the ESPGHAN guidelines for the diagnosis of childhood CD in the Netherlands and presents the difference in incidence and clinical presentation of CD in the Netherlands over the last 40 years. **Chapter 3** shows an overview of the current knowledge of the preventive strategies for CD. In the following two chapters, results of secondary prevention strategies are presented. **Chapter 4** shows the protocol of the case finding study GLUTENSCREEN: a prospective study to detect CD in young children attending the Preventive Youth Health Care Centers in the region Kennemerland for a regular visit. **Chapter 5** presents our developed and validated clinically useful prediction models for CD development among genetically predisposed children from celiac families and the application to provide individualized screening advice. The results are based on data from the long-term follow up of the PreventCD cohort. The PreventCD study evaluates the influence of infant feeding on the development of childhood CD and explored the possibility of inducing tolerance to gluten.

Clinical follow-up of children and adolescents with CD is necessary but evidence concerning the content of the follow up, as well as the frequency, is lacking. The next two chapters assess how to manage the follow up of CD in children and adolescents. Since the GFD is currently the only effective treatment of CD, assessment of dietary-adherence is important during the follow up of CD patients. A relatively new method for monitoring dietary compliance is the detection of GIP. **Chapter 6** presents the features

of GIP in urine during a consultation on the outpatient clinic. Children with CD visit the outpatient clinic for their follow up, but communication over the internet offers new opportunities. E-health has shown beneficial effects on the costs and quality of other chronic disease management, but the evidence of E-health in CD follow-up has not been systematically reviewed. Finally, **Chapter 7** shows the results of the systematic review of the current knowledge of E-health for the follow-up in CD patients. In **Chapter 8**, the main findings of this thesis are discussed in the light of the current literature, followed by the discussion and conclusion in Dutch in **Chapter 9**.

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabo IR et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr. 2012:54:136-60.
2. Singh P, Arora A, Strand TA, et al. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2018 Jun;16(6):823-836.
3. Catassi C, Kryszak D, Bhatti B, et al. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. Ann Med. 2010 Oct;42(7):530-8
4. Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of celiac disease over time. Aliment Pharmacol Ther. 2007; 26:1217–25.
5. Rubio-Tapia A, Ludvigsson JF, Brantner TL, et al. The prevalence of celiac disease in the United States. Am J Gastroenterol. 2012 Oct;107(10):1538-44;
6. Catassi C, Gatti S, Fasano A. The new epidemiology of celiac disease. J Pediatr Gastroenterol Nutr. 2014 Jul;59 Suppl 1:S7-9.
7. Bai J, Ciacci C. The World Gastroenterology Organisation Global Guidelines recommend testing for CD in asymptomatic children who have first-degree relatives with the disease. J Clin Gastroenterol. Volume 51, number 9. Febr 2017.
8. Ludvigsson JF, Rubio-Tapia A, Van Dyke CT, et al. Increasing incidence of celiac disease in a North American population. Am J Gastroenterol. (2013) 108:818–24.
9. George EK, Mearin ML, van der Velde EA, et al. Low incidence of childhood celiac disease in The Netherlands. Pediatr Res. 1995 Feb; 37(2):213-8
10. Steens RFR, Csizmadia CGDS, George EK, 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. 2005 Aug; 147(2):239-43.
11. Vavricka SR, Vadasz N, Stotz M, et al. Celiac disease diagnosis still significantly delayed—doctor's but not patients' delay responsive for the increased total delay in women. Dig Liver Dis. 2016;48:1148–54.
12. Csizmadia CGDS, Mearin ML, Blomberg BM, et al. An iceberg of childhood celiac disease in the Netherlands. Lancet 1999;353:813-4.
13. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: am involving spectrum. Gastroenterology. 2001; 120:636–51.
14. Mearin ML. Celiac disease among children and adolescents. Curr Probl Pediatr Adolesc Health Care. 2007; 37:86–105.
15. Kiefte-de Jong JC, Jaddoe VW, Uitterlinden AG, et al. Levels of antibodies against tissue transglutaminase during pregnancy are associated with reduced fetal weight and birth weight. Gastroenterology. 2013;144:726–35.
16. Green PHR, Jabri B. Celiac disease. Lancet. 2003; 362:383–91.
17. Werkstetter KJ, Korponay-Szabó IR, Popp A, et al. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology. 2017;153(4):924-935.
18. Korponay-Szabo IR, Szabados K, Pusztai J, et al. Population screening for celiac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study. BMJ. 2007;335:1244-7.
19. Vriezinga S, Borghorst A, van den Akker-van Marle E, et al. E-healthcare for celiac disease–a multicenter randomized controlled trial. J Pediatr. 2018;195:154–60.

20. Myléus A, Reilly NR, Green PHR. Rate, Risk Factors, and Outcomes of Nonadherence in Pediatric Patients With Celiac Disease: A Systematic Review. Clin Gastroenterol Hepatol; 2020;18(3):562-73
21. Wessels MMS, Te Linterlo M, Vriezinga SL, et al. Assessment of dietary compliance in celiac children using a standardized dietary interview. Clin Nutr. 2018;37 (3): 1000-4.
22. Green P, Jabri B, Celiac Disease. Annu. Rev. Med. 2006. 57:207–21
23. Koning F, Schuppan D, Cerf-Bensussan N, et al. Pathomechanisms in celiac disease. Best Prac Res Clin Gastro 2005 June;19(3):373-87
24. Mearin ML. Celiac disease among children and adolescents. Curr Probl Pediatr Adolesc Health Care. 2007;37:86–105.
25. Hogen Esch CE, Wolters VM, Gerritsen SA, et al. Specific celiac disease antibodies in children on a gluten-free. Pediatrics; 2011; 128:547–552.
26. Husby S, Murray JA, Katzka DA. AGA Clinical Practice Update on Diagnosis and Monitoring of Celiac Disease-Changing Utility of Serology and Histologic Measures: Expert Review. Gastroenterology. 2019 Mar;156(4):885-889.
27. Silvester JA, Kurada S, Szwajcer A, et al. Tests for Serum Transglutaminase and Endomysial Antibodies Do Not Detect Most Patients With Celiac Disease and Persistent Villous Atrophy on Gluten-free Diets: a Meta-analysis. Gastroenterology. 2017 Sep;153(3):689-701.
28. Moreno M, Rodríguez-Herrera A, Sousa C, et al. Biomarkers to Monitor Gluten-Free Diet Compliance in Celiac Patients. Nutrients. 2017;9(1):46
29. Gerasimidis K., Zafeiropoulou K., Mackinder M., et al. Comparison of Clinical Methods With the Faecal Gluten Immunogenic Peptide to Assess Gluten Intake in Celiac Disease. Journal of Pediatric Gastroenterology and Nutrition, 2018; 67(3), 356–360.
30. Comino I, Fernández-Bañares F, Esteve M, et al. Fecal Gluten Peptides Reveal Limitations of Serological Tests and Food Questionnaires for Monitoring Gluten-Free Diet in Celiac Disease Patients. American Journal of Gastroenterology. 2016;111(10):1456-1465.
31. Comino I, Segura V, Ortigosa L, et al. Prospective longitudinal study: use of faecal gluten immunogenic peptides to monitor children diagnosed with celiac disease during transition to a gluten-free diet. Aliment Pharmacol Ther. 2019 Jun;49(12):1484-1492.
32. Stefanolo JP, Tálamo M, Dodds S, et al. Real-World Gluten Exposure in Patients With Celiac Disease on Gluten-Free Diets, Determined From Gliadin Immunogenic Peptides in Urine and Fecal Samples. Clin Gastroenterol Hepatol. 2021;19(3):484-91.
33. Silvester JA, Comino I, Rigaux LN, et al. Exposure sources, amounts and time course of gluten ingestion and excretion in patients with celiac disease on a gluten-free diet. Aliment Pharmacol Ther 2020 Nov;52(9):1469-79.
34. Richtlijn Coeliakie en Dermatitis Herpetiformis. Haarlem: Nederlandse Vereniging voor Maag-Darm-Leverartsen 2008.
35. Maars van der PJ, Mackenbach JP. Volksgezondheid en Gezondheidszorg. Elsevier; Bunge. 1999. Tweede druk [Dutch].
36. Meijer C, Shamir R, Szajewska H, et al. Celiac disease prevention. Front Pediatr 2018;6:368.
37. Vriezinga SL, Schweizer JJ, Koning F, et al. Celiac disease and gluten-related disorders in childhood. Nat Rev Gastroenterol Hepatol. 2015; 12(9):527-36.

2

Efficient implementation of the ‘non-biopsy approach’ for the diagnosis of childhood celiac disease in the Netherlands. A national prospective evaluation 2010-2013

Eur J Pediatr 2021 Aug;180(8):2485-2492

Caroline R. Meijer
Joachim J. Schweizer
Anne Peeters
Hein Putter
M. Luisa Mearin

ABSTRACT

The aim of this study was 1) to prospectively evaluate the nationwide implementation of the ESPGHAN-guidelines for the diagnosis of celiac disease (CD), 2) to investigate the incidence and clinical presentation of diagnosed childhood CD (0-14 years) in the Netherlands and 3) to compare the findings with national survey data from 1975-1990 and 1993-2000 using the same approach. From 2010 to 2013, all practicing pediatricians were invited to report new celiac diagnoses to the Dutch Pediatric Surveillance Unit. Data were collected via questionnaires. 1107 Children with newly diagnosed CD were reported (mean age: 5.8 years; range: 10 months-14.9 years; 60.5% female). After the introduction of the non-biopsy approach in 2012, 75% of the diagnoses were made according to the guideline with a significant decrease of 46.3% in biopsies. The use of EMA and HLA-typing significantly increased with 25.8% and 62.1%, respectively. The overall incidence rate of childhood CD was 8.8-fold higher than in 1975-1990 and 2.0-fold higher than in 1993-2000. During the study period, the prevalence of diagnosed CD was 0.14%, far below 0.7% of CD identified via screening in the general Dutch pediatric population. Clinical presentation has shifted towards less severe and extra-intestinal symptoms. *Conclusion:* ESPGHAN guidelines for CD diagnosis in children were effectively and rapidly implemented in the Netherlands. Incidence of diagnosed CD among children is still significantly rising with a continuous changing clinical presentation. Despite the increasing incidence of diagnoses, significant underdiagnosis still remains.

INTRODUCTION

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamins in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy (1). Up until a few decades ago, CD was considered an uncommon disease that mainly affected children and limited to Western Europe. However, the current prevalence of CD in the general population is estimated to be approximately 1% in different parts of the world (2, 3).

In the Netherlands, two national surveys on CD diagnosed in childhood performed by our group between 1975-1990 (retrospective) and 1993-2000 (prospective), showed that the incidence of diagnoses increased significantly from 0.18/1000 to 0.81/1000 live-births, respectively (4, 5). However, as also reported in other countries (6, 7), this increase in the incidence of diagnoses did not correspond nearly as much with the prevalence of CD detected by screening in the overall pediatric population (8, 9), indicating that CD was heavily underdiagnosed in the Netherlands. Our previous Dutch surveys showed that the clinical presentation in children had also shifted towards more subtle symptoms (4, 5). The results of our prospective study from 1993-2000 were based on data from the Dutch Pediatric Surveillance Unit (DPSU) comprising all Dutch pediatric practices, with a mean response rate of 90% (2010). The CD diagnoses were cross-checked by reviewing the National Database of Pathology (in Dutch: Pathologisch Landelijk Geautomatiseerd Archief – PALGA), to identify all biopsy-proven CD cases according to the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) 1990 diagnostic criteria (10). In 2012, ESPGHAN published new diagnostic guidelines with the so-called "non-biopsy diagnostic approach" for symptomatic children suspected of CD (1). Nevertheless, novel diagnostic guidelines are not always effectively implemented in daily practice (11).

The aims of the present study are to (i) prospectively evaluate the nationwide implementation of the ESPGHAN guidelines 2012 for CD diagnosis in the Netherlands and, (ii) investigate the incidence and clinical presentation of diagnosed childhood CD from 2010-2013 in the Netherlands in comparison to previous national surveys.

METHODS

A four-year prospective observational cohort study, including all children aged 0 -14 years and diagnosed with CD throughout the Netherlands between January 1st, 2010, and December 31st, 2013, as reported to the DPSU. The purpose of the DPSU of the Dutch Society of Pediatrics (DSP) is to gain insight on the prevalence of diseases in youths (0-18 years) on a population level and to promote scientific research addressing the background, nature, prognosis, treatment and prevention of these diseases (11). All Dutch pediatricians were asked by paper (until 2010) or through an internet-based system to report new cases of selected conditions, for our study CD, on a monthly basis, followed immediately or later by completing a questionnaire. This questionnaire, which was filled in by the pediatrician, collected patient information such as gender, age, parents' country of origin, symptoms at presentation, anthropometrics (height and weight), associated diseases, family history and (results of) diagnostic tests. Personal data were limited to initials and birth dates to guard patient confidentiality. The completed questionnaires were subsequently sent to the investigators of the Leiden University Medical Centre (LUMC) where the data were stored and analysed. In December 2013, registration was unintentionally closed due to relocation of the DPSU to another organization. Up until 2012, data from the DPSU were cross-checked using information provided by PALGA, the database that anonymously registers all pathological specimens collected in the Netherlands (including sex, age, date of biopsy). The primary outcome comprised the diagnostic workup before and after the introduction of the non-biopsy diagnostic approach in 2012. The secondary outcome was the clinical presentation compared to that from previous surveys and the incidence of diagnosed CD in the Netherlands from 2010-2013 in children aged 0-14 years as the numerator and the number of live-births in these years as the denominator, expressed as a rate per 1,000 live-births. The age of the included children (0-14 years) and the metrics were chosen with the purpose to be able to compare the results to those reported in our previous surveys. Patient information was completely anonymized and guaranteed throughout the study.

The ethical aspects have been approved by the DPSU of the DSP in accordance with the applicable rules on privacy. According to Dutch Law for the use of completely anonymous data informed consent is not needed.

Statistical analysis

All categorical data are described as frequencies. Percentages are based on the total number of included patients.

For the incidence rate we used the data from all the reported children and for the analysis of the clinical picture and the diagnostic work up we used the data from the children with completed questionnaires. Demographic and epidemiological data regarding the general population were provided by The Dutch Central Bureau of Statistics (CBS, The Hague, the Netherlands) (12). The emigration and immigration rates per 1000 inhabitants in the Dutch population remained stable during the study period (2010 and 2013: 1.2 and 1.1) (13).

The diagnostic approach, incidence rates and clinical presentation of CD in 2010-2013 were compared to the data from 1975-1990 and 1993-2000 using the Chi-square test and Chi-square test for trend. A p-value of 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 23.0.

RESULTS

From January 1st, 2010, to December 31st, 2013, 1325 children with CD were reported to the DPSU, 218 of which were excluded (78 older than 15 years at diagnosis; 123 double reported; 11 withdrawn by pediatrician; 6 diagnosed outside the study period). Of the 1107 included patients (mean age: 5.8 years; range: 10 months-14.9 years; 60.5% female), 209 were only reported as new CD diagnosis and from the additional 898 completed questionnaires were returned. The mean survey response rate of Dutch pediatricians to the monthly CD request was 81.1%, of which 87.1%, 84.7%, 77.4% and 74.1% pertained to the years 2010, 2011, 2012 and 2013, respectively.

Diagnostic approach

The diagnostic approach is summarised in Table 1. Utilization of the anti-gliadin antibodies- (AGA) and endomysium antibodies (EMA) -tests decreased significantly over the period 1993-2000 and 2010-2013 from 90% (n=915) to 9.4% (n=84) ($p<0.001$) and from 78.0% (n=793) to 60.5% (n=543) ($p<0.001$), respectively. In contrast, the use of the EMA-test increased from 48.7% (n=237) in 2010-2011 to 74.5% (n=306) in 2012-2013 ($p<0.001$). This was also the case for HLA-typing which increased significantly from 23.8% (n=116) in 2010-2011 to 85.9% (n=353) in 2012-2013 ($p<0.001$). Anti-tissue transglutaminase antibodies (tTG) levels were determined in the majority of children (96.8%, n=869) diagnosed in 2010-2013. Moreover, in this last period, 66.9% (n=601) children underwent diagnostic small bowel biopsies which showed a significant decrease from 88.1% (n=429) to 41.8% (n=172) ($p<0.001$) after the publication of the non-biopsy ESPGHAN guideline in 2012 (1) (Table 1).

Table 1. Changing Diagnostic Work Up for Celiac Disease in Children in The Netherlands. On the left side the data from the three national surveys are presented (1975-2013) and on the right side the data before and after the introduction of the non-biopsy approach.

Diagnostic tests No. (%)	National Surveys			2010-2013			
	1975-1990 Retrospective	1993-2000 Prospective	2010-2013 Prospective	2010-2011		2012***-2013	
	n=223	n=1017	n = 898	n = 487		n = 411	
				Sympt. n=454	**Asympt. n=33**	**Sympt. n=384**	**Asympt. n=27**
IgA AGA	**131 (59)**	**915 (90)**	**84 (9.4)***	45 (9.9)	0	39 (10.2) n.s.	0 n.s.
				45 (9.2)		**39 (9.5)** n.s.	
IgA tTG	**N.A.****	**N.A.****	**869 (96.8)**	440 (96.9)	33 (100)	370 (96.4) n.s.	26 (96.3) n.s.
				473 (97.1)		**396 (96.4)** n.s.	
IgA EMA	**Unknown**	**793 (78)**	**543 (60.5)***	223 (49.1)	14 (42.4)	288 (75.0)^	18 (66.7)^
				237 (48.7)		**306 (74.5)***	
HLA typing	**Unknown**	**Unknown**	**469 (52.2)**	107 (23.6)	9 (27.3)	329 (85.7)^	24 (88.9)^
				116 (23.8)		**353 (85.9)^**	
Biopsies	**223 (100)**	**1017 (100)**	**601 (66.9)***	399 (87.9)	30 (90.1)	150 (39.1)^	22 (81.5) n.s.
				429 (88.1)		**172 (41.8)^**	

*p<0,01; NA= not available at the time; **Widespread introduction throughout the Netherlands in 1999; ***Publication of ESPGHAN Guideline. Comparison of data between 2010-2011 and 2012-2013: n.s.= not significantly different or ^= significantly different

In total, 411 children were newly diagnosed with CD in 2012-2013. From them 93.4% (n=384) was symptomatic and 6.6% (n=27) was asymptomatic. 234 Of the symptomatic children had tTG levels ≥10x upper limit of normal (ULN) and were eligible for the non-biopsy approach; more than 75% (58/234) of the children were correctly diagnosed according to the guideline. Of all symptomatic children 77.3% (297/384) were correctly diagnosed as well as 81.5% (22/27) of the asymptomatic children. So, a total of 77.6% of the children (319/411) were correctly diagnosed according to the new ESPGHAN algorithms. Reasons for incorrect application of the ESPGHAN guidelines of 2012 regarding the symptomatic algorithm (in which data were missing for 2 children) were presence of Marsh classification score of 0-1 (n = 11; 12.6%) and include missing EMA, HLA-typing and tTG-tests in 46 (52.9%), 21 (24.1%) and 7 (8.0%) children, respectively. In 5 children, the reasons for incorrect application of the asymptomatic algorithm (in which data were missing for 2 children) were refusal to undergo diagnostic biopsies (n = 3; 60.0%).

Frequency rates

Figure 1 details the significantly higher crude incidence rate of diagnosed CD in 2010-2013 (1.59/1000 live-births) as compared to the previous studies from 1975-1990 and 1993-2000, which report incidences of 0.18 and 0.81 per 1000 live-births, respectively (4,5) (p<0.001). The reported crude incidence rate of diagnosed CD in the present study

was 1.51 per 1000 live-births in 2010; 1.60 in 2011; 1.86 in 2012 and 1.35 in 2013 (Figure 1). The prevalence of diagnosed CD in 2010-2013 was 0.14%, which is significantly lower than the 0.5% detected by screening of the general Dutch pediatric population of 2-4-year-olds reported in 1999 and 0.7% of 6-year-olds reported in 2015 (p<0.001) (8, 9).

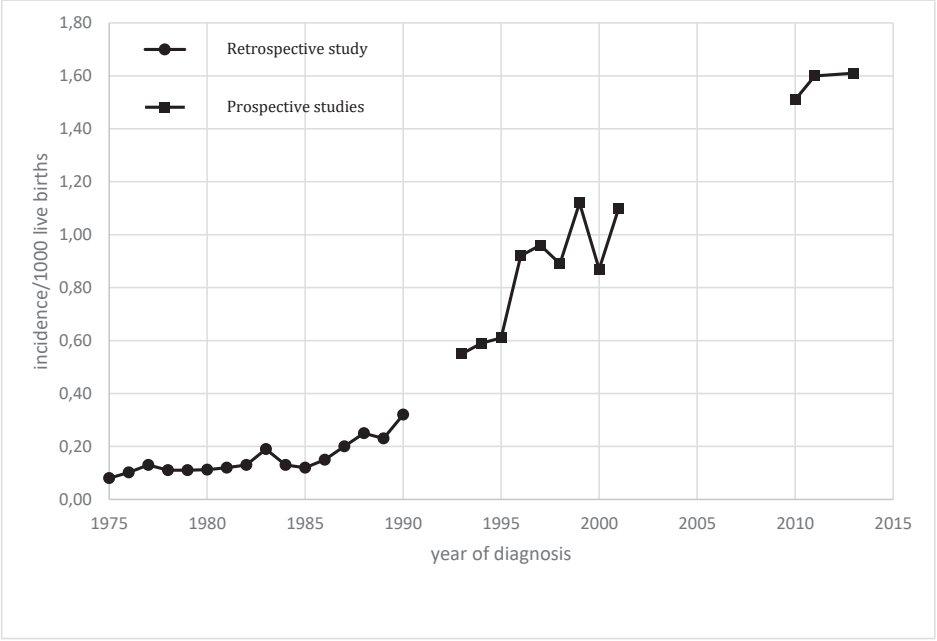


Figure 1. Incidence of diagnosed childhood celiac disease in three national studies in the Netherlands (n=223 in 1975-1990; n=1017 in 1993-2000 and n=1107 in 2010-2013)

Clinical presentation

Characteristics of the reported CD patients are presented in Supplementary Table 1. Parents of 92.2% of children (n=828) reported one or more CD-related symptoms at the time of diagnosis, with abdominal pain, wasting (defined as weight <p10) and stunting (height for age < p10) being the most frequently reported symptoms at 49.6% (n=445), 33.9% (n=304) and 32.0% (n=287), respectively (Supplementary Table 1). Only 36 patients (4.0%) presented with the classical triad, i.e., chronic diarrhoea, abdominal distension and failure to thrive. At least 1 gastrointestinal symptom was reported in 669 (74.5%) patients, while 149 (16.6%) exclusively experienced extra-intestinal symptoms.

Table 2 shows the continuous and significantly changing clinical presentation of diagnosed CD in comparison to the presentation reported in 1975-1990 and in 1993-2000. Although there is a significant decrease in chronic diarrhoea and abdominal distension as presenting symptoms, significantly more children presented with abdominal pain,

lassitude and anorexia. Thirty percent of the children were ≤ 2 years of age, which was significantly younger than reported by the previous surveys. In total, 13.8% of the children had a first degree relative (FDR) with CD, while only 7.0% of them were referred to the pediatrician for screening based on a positive family history for CD.

Table 2. Comparison of the Clinical Presentation of Diagnosed Childhood Celiac Disease in Three National Surveys in the Netherlands

	1975-1990 % (n=223)	1993-2000 % (n=1017)	2010-2013 % (n=898)	P value
Chronic diarrhoea	72	41	25	<0.01
Abdominal distension	76	48	28	<0.01
Growth failure in height and weight	63	24	19	<0.01
Weight for height < P10	22	49	34	<0.01
Height for age < P10	42	34	32	<0.01*
Abdominal pain	7	16	50	<0.01
Lassitude	Not known	12	24	<0.01
Anorexia	0	5	24	<0.01
Age ≤ 2 yr.	60	47	30†	<0.01
Median age (yr.)	1.5	2.1	5.8 †	<0.01

* Comparison of data only significantly different between 1975-1990 and 2010-2013.
† Age of all 1107 reported CD children.

DISCUSSION

In 2012, ESPGHAN published new guidelines for the diagnosis of CD in children and adolescents, including the novel so-called “non-biopsy approach” for selected cases (1). Our national prospective data show that in 2012-2013, childhood CD was diagnosed in the Netherlands according to the new guidelines in more than 75% of the cases, with 75.2% correct application of the ‘non-biopsy’ approach, indicating a quick and efficient implementation of the new guidelines. Such successful implementation does not always follow the publication of novel guidelines (11). For example, after the publication of the guideline for the diagnosis and management of gastroesophageal reflux in children, only 1.8% of the general pediatricians showed complete adherence to it (11); a frequency that increased to 46.1% after specific training (14).

The effective implementation in the Netherlands has possibly been facilitated first because they were actively overtaken by the DSP immediately following their publication, and second because of the extended use of the highly sensitive tTG-test which is imperative in the 2012 ESPGHAN diagnostic guidelines (1, 15) (Table 1). The variable use of the EMA-test, both in the Netherlands and in other countries (16), is explained

by the introduction of the more simple and economical tTG-test in the 1990's, followed by an increase in its use after the publication of the ESPGHAN guidelines of 2012 in which its determination was established for the initial diagnostic work-up and for the confirmation of CD diagnoses under the non-biopsy approach (1, 17, 18). The use of the EMA-test as a confirmatory diagnostic test has been reinforced by the updated ESPGHAN guidelines of 2020, so an increase in its implementation may be expected in the future, particularly in children diagnosed without biopsies (19). The significant reduction (46.3%) of diagnostic biopsies in our country, which is in accordance with findings from other studies (16, 20), indicates that the implementation of the non-biopsy strategy has taken place quickly and efficiently. However, the guidelines for non-biopsy diagnosis in children have not yet been adopted in all countries, despite its numerous advantages such as the reduction in medical costs and avoidance of general anaesthesia or deep sedation (21, 22). With the conditional recommendation of the non-biopsy approach in the ESPGHAN guidelines of 2020 for asymptomatic children, a further decline in small bowel biopsies is to be expected.

Our data show a continuous and significant 8.8-fold increase in the incidence of CD diagnosed in childhood in the Netherlands from 1975 to 2013, with a 2.0-fold increase from 1993-2000 to 2010-2013. This is in accordance with the 2.4 -fold increase found in the retrospective nationwide survey on newly diagnosed CD both in children and adults from 1995-2010 (23). Our results also agree with the findings from recent European and Canadian studies conducted in pediatric populations which likewise show an increasing trend over time in the frequency of clinically diagnosed childhood CD (24-26). The rising incidence in the number of diagnoses is likely caused by a combination of several factors, namely, the growing awareness of CD among healthcare professionals, increased screening of high-risk groups and the availability of reliable CD antibody tests (1), but also a true rise in the incidence of CD (27). In this respect, an increasing prevalence of CD has been shown in screening studies among school-aged children with a 1.4-fold increase over a period of 15 years in the Netherlands and over 1.8-times in 25 years in Italy (8,9,28). This is in line with the 5-fold increase in prevalence of CD autoimmunity over a period of 50 years found in the United States, a finding based on the analysis of stored sera from community subjects compared with sera collected at an earlier date (29). In contrast, no increase over time in the prevalence of CD has been reported in adult blood donors in Israel (30).

Strengths of our study include the reporting of national data which forms a seamless representation for the whole country of the Netherlands, as well as utilization of the same methods as in the survey performed in 1993-2000, improving the reliability of the result comparisons. Nevertheless, a possible limitation of our study is the decreased response

rate to the DPSU monitoring system (from 87.1% in 2010 to 74.1% in 2013 versus 90% in 1993-2000) (5). This decrease is possibly due to the overall increasing administrative burden, complexity of care and reduced time for reporting among Dutch pediatricians as well as the relocation of the DPSU to another organisation at the end of 2013 (31). The relatively low response rate of 2013, which does not represent a true decline in the incidence of CD diagnoses, is the most plausible cause of the abrupt decrease in the incidence of CD diagnoses reported to the DPSU in this year when compared to previous years, even after correcting for the preliminary closing of the reporting system.

Our findings of a continuously changing clinical presentation and significant increase in the median age at diagnosis are in agreement with those reported by other countries (16-19, 32-38). The actual clinical presentation of CD diagnosed in childhood in the Netherlands is formed by a variable combination of abdominal pain and poor growth (in weight or in height). The classical triad of diarrhoea, abdominal distension and failure to thrive is rare although each of these symptoms is present in many CD patients (Supp Table 1) (39-40). Failure to thrive (defined as height for age <p10 and weight for height <p10) occurs significantly less frequent than before (44%), even though the absolute frequency has remained fairly stable over the time (n=140 in 1975-1990, n=244 in 1993-2000 and n=166 in the present study). Interestingly, 70% of the diagnosed children in 2010-2013 had at least one non-gastrointestinal symptom, with lassitude and anorexia also increasing significantly (Table 1) (4, 5). The shift in CD presenting symptoms towards a milder form of disease may also potentially be the reason for an upward shift of age at diagnosis (39-40).

In conclusion, the ESPGHAN guidelines 2012 for the diagnosis of CD in children were effectively and quickly implemented in the Netherlands. During the 2 years after their publication, the guidelines were applied in more than 75% of the cases, particularly in older children. The clinical presentation of childhood CD in the Netherlands is characterised by a continuous change with a shift towards less severe and non-gastrointestinal symptoms. The incidence of diagnosed CD in childhood from 2010-2013 in the Netherlands has increased significantly by 8.8-fold from 1975-1990 and 2.0-fold from 1993-2000. Despite the rising incidence in the number of diagnoses, the prevalence of diagnosed CD is significantly lower that the prevalence of disease identified by screening, signifying that childhood CD is still significantly underdiagnosed in the Netherlands.

Supplementary table 1. Characteristics of 898 children with celiac disease diagnosed in 2010-2013 as reported to the Dutch Pediatric Surveillance Unit

	2010 n = 243	2011 n = 244	2012 n = 254	2013 n = 157	Total n = 898
Median age at diagnosis of CD, in years	5.7	6.0	5.8	6.0	**5.8**
Age ≤ 2, in %	29.7	28.5	32.0	29.2	
Female, No. (%)	166(68.3)	159 (65.2)	139 (54.7)	79 (50.3)	**543 (60.5)**
Unknown	6 (2.5)	12 (4.9)	22 (8.7)	21 (13.4)	**61 (6.8)**
Reason for referral, No. (%)					
Suspected CD	188(77.4)	201 (82.4)	209 (82.3)	121 (77.1)	**719 (80.1)**
Positive family history	18 (7.4)	17 (7.0)	17 (6.7)	11 (7.0)	**63 (7.0)**
Associated disease	20 (8.2)	22 (9.0)	22 (8.7)	19 (12.1)	**83 (9.2)**
Suspected CD + positive family history	2 (0.8)	0 (0)	0 (0)	2 (1.9)	**5 (0.6)**
Suspected CD + associated disease	2 (0.8)	0 (0)	0 (0)	0 (0)	**2 (0.2)**
Unknown	13 (5.3)	4 (1.6)	6 (2.4)	3 (1.9)	**26 (2.9)**
Symptoms, No. (%)					
No symptoms	20 (8.2)	23 (9.4)	17 (6.7)	10 (6.4)	**70 (7.8)**
Anorexia	59 (24.3)	53 (21.7)	64 (25.2)	39 (24.8)	**215 (23.9)**
Recurrent oral ulcers	5 (2.1)	4 (1.6)	1 (0.4)	1 (0.6)	**11 (1.2)**
Nausea	8 (3.3)	10 (4.1)	18 (7.1)	14 (8.9)	**50 (5.6)**
Vomiting	25 (10.3)	32 (13.1)	28 (11.0)	17 (10.8)	**102 (11.4)**
Abdominal pain	104(42.8)	113 (46.3)	137 (53.9)	91 (58.0)	**445 (49.6)**
Abdominal distension	69 (28.4)	69 (28.3)	72 (28.3)	39 (24.8)	**249 (27.7)**
Constipation	45 (18.5)	46 (18.9)	56 (22.0)	30 (19.1)	**177 (19.7)**
Acute diarrhoea (<15 days)	6 (2.5)	7 (2.9)	7 (2.8)	8 (5.1)	**28 (3.1)**
Chronic diarrhoea (>4 weeks)	61 (25.1)	55 (22.5)	68 (26.8)	36 (22.9)	**220 (24.5)**
Pallor	28 (11.5)	33 (13.5)	26 (10.2)	8 (5.1)	**95 (10.6)**
Lassitude	55 (22.6)	67 (27.5)	65 (25.6)	28 (17.8)	**215 (23.9)**
Irritability	37 (15.2)	41 (16.8)	54 (21.3)	26 (16.6)	**158 (17.6)**
Delayed puberty	2 (0.8)	3 (1.2)	0 (0)	0 (0)	**5 (0.6)**
Joint disorders	3 (1.2)	1 (0.4)	1 (0.4)	1 (0.6)	**5 (0.6)**
Failure to thrive	41 (16.9)	47 (19.3)	50 (19.7)	28 (17.8)	**166 (18.5)**
Wasting (Weight for height < P10)	79 (32.5)	79 (32.4)	97 (38.2)	49 (31.2)	**304 (33.9)**
Stunting (Height for age < P10)	74 (30.5)	88 (36.1)	73 (28.7)	52 (33.1)	**287 (32.0)**
Non-gastrointestinal symp	191(68.5)	186 (64.6)	238 (72.6)	161 (75.9)	**776 (70.1)***
Unknown	13 (5.3)	2 (0.8)	4 (1.6)	1 (0.6)	**18 (2.0)**
Associated disease, No. (%)	28 (11.5)	29 (11.9)	31 (12.2)	19 (12.1)	**107 (11.9)**
Type 1 Diabetes	20 (8.2)	9 (3.7)	16 (6.3)	12 (7.6)	**57 (6.3)**
Down Syndrome	3 (1.2)	15 (6.1)	12 (4.7)	5 (3.2)	**35 (3.9)**
Turner Syndrome	1 (0.4)	-	-	-	**1 (0.1)**
Selective IgA Deficiency (0.05 g/l)	3 (1.2)	3 (1.2)	2 (0.8)	1 (0.6)	**9 (1.0)**
Other#	1(0.4)	2 (0.8)	1 (0.4)	1 (0.6)	**5 (0.6)**
Unknown	12 (4.9)	6 (2.5)	10 (3.9)	3 (1.9)	**31 (3.5)**
Relative with CD, No (%)	37(15.2)	31(12.7)	34 (13.4)	22(14.0)	**124 (13.8)**

*149 children had exclusively non-gastrointestinal symptoms. #Rheumatoid Arthritis and Autoimmune Thyroiditis

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al (2012) European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr. 54(1):136–60
2. Vriezinga SL, Schweizer JJ, Koning F, Mearin ML (2015) Celiac disease and gluten-related disorders in childhood. Nat Rev Gastroenterol Hepatol. 12(9):527-36.
3. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al (2018) Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Volume 16, Issue 6, pp 823–836.
4. George EK, Mearin ML, van der Velde EA, Houwen RH, Bouquet J, Gijsbers CF, et al (1995) Low incidence of childhood celiac disease in The Netherlands. Pediatr Res 37(2):213-8
5. Steens RF, Csizmadia CG, George EK, Ninaber MK, Hira Sing RA, Mearin ML (2005) A national prospective study on childhood celiac disease in the Netherlands 1993-2000: an increasing recognition and a changing clinical picture. J Pediatr 147(2):239-43.
6. Rosén A, Sandström O, Carlsson A, Högberg L, Olén O, Stenlund H, et al (2014) Usefulness of Symptoms to Screen for Celiac Disease? Pediatrics. 133 (2) 211-218
7. Hujoel IA, Van Dyke CT, Brantner T, Larson J, King KS, Sharma A, et al (2018) Natural history and clinical detection of undiagnosed celiac disease in a North American community. Aliment Pharmacol Ther. 47(10): 1358–1366.
8. Csizmadia CG, Mearin ML, von Blomberg BM, Brand R, Verloove-Vanhorick SP (1999) An iceberg of childhood celiac disease in the Netherlands. Lancet. 353(9155):813-4.
9. Jansen M, van Zelm M, Groeneweg M, Jaddoe V, Dik W, Schreurs M, et al (2018) The identification of celiac disease in asymptomatic children: the Generation R Study. J Gastroenterol. 53(3):377-386.
10. Revised criteria for diagnosis of celiac disease: report of Working Group of European Society for Pediatric Gastroenterology and Nutrition (1990) Arch Dis Child 65:909-11.
11. Quitadamo P, Papadopoulou A, Wenzl T, et al (2014) European pediatricians' approach to children with GER symptoms: survey of the implementation of 2009 NASPGHAN-ESPGHAN guidelines. J Pediatr Gastroenterol Nutr. 58(4):505-9.
12. Hira Sing RA, Rodrigues Pereira R (2002) Het Nederlands Signaleringscentrum Kindergeneeskunde; een kwaliteitsinstrument voor preventie en onderzoek. Ned Tijdschr Geneeskd 146:2409-14.
13. Dutch Central Bureau of Statistics 2010-2013. http://www.cbs.nl.
14. Quitadamo P, Urbonas V, Papadopoulou A et al (2014) Do pediatricians apply the 2009 NASPGHAN-ESPGHAN guidelines for the diagnosis and management of gastroesophageal reflux after being trained? J Pediatr Gastroenterol Nutr. 59(3):356-9.
15. Dieterich W, Ehnis T, Bauer M, et al (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. Nature Med 7: 797—801
16. Werkstetter KJ, Korponay-Szabó IR, Popp A, et al (2017) Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology. 153(4):924-935.
17. Giersiepen K, Lelgemann M, Stuhldreher N, et al (2012). Accuracy of diagnostic antibody tests for celiac disease in children: summary of an evidence report. J Pediatr Gastroenterol Nutr 54(2):229-41
18. Zevit N, Shamir R (2014) Diagnosis of celiac disease: where are we heading after the ESPGHAN 2012 guidelines? J Pediatr Gastroenterol Nutr 59 Suppl 1:S13-S15.

19. Husby S, Koletzko S, Korponay-Szabó I et al (2020). European Society Pediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Celiac Disease 2020. J Pediatr Gastroenterol Nutr. 70(1):141-156.
20. Donat E, Ramos JM, Sánchez-Valverde F, et al (2016). ESPGHAN 2012 Guidelines for Celiac Disease Diagnosis: Validation Through a Retrospective Spanish Multicentric Study. J Pediatr Gastroenterol Nutr. 62(2):284-91.
21. Ermarth A, Bryce M. Woodward S, et al (2017) Identification of Pediatric Patients With Celiac Disease Based on Serology and a Classification and Regression Tree Analysis. Clin Gastroenterol Hepatol 15:396-402.
22. Rajani S, Huynh HQ, Shirton L, et al (2016) A Canadian Study toward Changing Local Practice in the Diagnosis of Pediatric Celiac Disease. Can J Gastroenterol Hepatol. doi: 10.1155/2016/6234160
23. Burger JP, Roovers EA, Drenth JP, Meijer JW, Wahab PJ (2014) Rising incidence of celiac disease in the Netherlands; an analysis of temporal trends from 1995 to 2010. Scandinavian Journal of Gastroenterology. 49: 933–941.
24. Namatovu F, Sandström O, Olsson C, Lindkvist M, Ivarsson A (2014) Celiac disease risk varies between birth cohorts, generating hypotheses about causality: evidence from 36 years of population-based follow-up. BMC Gastroenterol 14:59.
25. Roma E, Panayiotou J, Karantana H, Constantinidou C, Siakavellas SI, Krini M, et al (2009) Changing pattern in the clinical presentation of pediatric celiac disease: a 30-year study. Digestion 80(3):185-91.
26. White LE, Merrick VM, Bannerman E, Russell RK, Basude D, Henderson P, et al (2013) The rising incidence of celiac disease in Scotland. Pediatrics 132:1–8
27. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, et al (2017) Increasing incidence and altered presentation in a population-based study of pediatric celiac disease in North America. J Pediatr Gastroenterol Nutr. 65:432–7.
28. Gatti S, Lionetti E, Balanzoni L, Verma AK, Galeazzi T, Gesuita R, et al (2019) Celiac Screening Team. Increased Prevalence of Celiac Disease in School-age Children in Italy. Clin Gastroenterol Hepatol. 18:596–603
29. Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, et al (2009) Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology 137: 88–93.
30. Levinson-Castiel R, Eliakim R, Shinar E, Perets TT, Layfer O, Levhar N, et al (2019) Rising prevalence of celiac disease is not universal and repeated testing is needed for population screening. United European Gastroenterol J. 7(3):412-418.
31. DPSU letter; https://www.nvk.nl/
32. Ress K, Luts K, Rägo T, Pisarev H, Uibo O (2012) Nationwide study of childhood celiac disease incidence over a 35-year period in Estonia. Eur J Pediatr 171(12):1823-8.
33. Iwanczak B, Matusiewicz K, Iwanczak F (2013) Clinical picture of classical, atypical and silent celiac disease in children and adolescents. Adv Clin Exp Med 22(5):667-73.
34. Ravikumara M, Tuthill DP, Jenkins HR (2006) The changing clinical presentation of celiac disease. Arch Dis Child 91(12):969-71.
35. Telega G, Bennet TR, Werlin S (2008) Emerging new clinical patterns in the presentation of celiac disease. Arch Pediatr Adolesc Med 162(2):164-8.
36. Garampazzi A, Rapa A, Mura S, Capelli A, Valori A, Boldorini R, et al (2007) Clinical pattern of celiac disease is still changing. J Pediatr Gastroenterol Nutr 45(5):611-4.37.
37. McGowan KE, Castiglione DA, Butzner JD (2009) The changing face of childhood celiac disease in north america: impact of serological testing. Pediatrics 124(6):1572-8.

38. Tapsas D, Hollén E, Stenhammar L, Fälth-Magnusson K (2016) The clinical presentation of celiac disease in 1030 Swedish children: changing features over the past four decades. Dig Liver Dis. 48:16–22.
39. Van Kalleveen MW., de Meij T., Plötz FB. Clinical spectrum of pediatric celiac disease: a 10-year single-centre experience. Eur J Pediatr. 2018 Apr;177(4):593-602.
40. Popp A, Mäki M (2019) Changing Pattern of Childhood Celiac Disease Epidemiology: Contributing Factors. Front Pediatr. 7:357.

3

Celiac Disease Prevention

Front Pediatr 2018 Nov 30;6:368.

Caroline R. Meijer
Raanan Shamir
Hania Szajewska
M. Luisa Mearin

ABSTRACT

Celiac disease (CD) is a common autoimmune disorder induced by ingestion of gluten in genetically susceptible individuals. Despite the prerequisite for a genetic predisposition, only a minority of the 40% of the Caucasian population that has this genetic predisposition develops the disease. Thus, environmental and/or lifestyle factors play a causal role in the development of CD. The incidence of CD has increased over the last half-century, resulting in rising interest in identifying risk factors for CD to enable primary prevention. Early infant feeding practices have been suggested as one of the factors influencing the risk of CD in genetically susceptible individuals. However, recent large prospective studies have shown that neither the timing of gluten introduction nor the duration or maintenance of breastfeeding influence the risk of CD. Also, other environmental influences have been investigated as potential risk factors, but have not led to primary prevention strategies. Secondary prevention is possible through early diagnosis and treatment. Since CD is significantly underdiagnosed and a large proportion of CD patients are asymptomatic at the time of diagnosis, secondary prevention will not identify all CD patients, as long as mass screening has not been introduced. As following a gluten-free diet is a major challenge, tertiary prevention strategies are discussed as well.

INTRODUCTION

The incidence and prevalence of celiac disease (CD) have risen over time; this is, in part, due to the current awareness in combination with the advent of highly sensitive and specific serological tests, but it also reflects a true increase in the prevalence of CD (1, 2). The clinical presentation of CD has changed dramatically in the last decades. Patients with atypical or non-specific symptoms often report a delay in diagnosis of CD that may last for years (3) or even worse, CD remains unrecognized and, therefore, untreated (4–6). Untreated disease is associated with long-term complications, such as chronic anemia, delayed puberty, neuropsychiatric disturbances, associated autoimmune disorders, infertility, small-for-date-births, osteoporosis, and, rarely, malignancy and it can reduce the quality of life (7–9). Treatment with a gluten-free diet (GFD) reduces the burden of morbidity and mortality associated with untreated CD. Thus, prevention would be beneficial (10). Prevention is defined as any activity that reduces the burden of mortality or morbidity from disease, taking place at the primary, secondary, or tertiary level (11) (Table 1). The purpose of this review is to present the current knowledge of the preventive strategies for CD (Table 2).

Table 1. Definition of levels of prevention

Primary	Secondary	Tertiary
Avoiding the development of a disease	Early detection and treatment	Reducing the impact of existing disease by improved treatment

Table 2. Some possible prevention strategies for celiac disease, as discussed in this review

Primary	Secondary	Tertiary
• Infant feeding - Breastfeeding - Breastfeeding at the time of gluten introduction - Timing of gluten introduction - Amount of gluten at the time of gluten introduction • (Intestinal) infections • Type of delivery • Antibiotics • Microbiota	• Case finding • Screening of high-risk groups • Mass screening	• Optimal adherence to the gluten-free diet • Gluten immunogenic peptides • Dietary interview • Dietary questionnaires • Serology/duodenal biopsies • Additional treatments - Larazotide acetate - Endopeptidases - Desensitization therapy

PRIMARY PREVENTION

Infant feeding

Theoretically, CD could be prevented by avoiding gluten introduction into the feeding of infants genetically predisposed to CD. However, this is not a realistic strategy, because the strongest genetic predisposing factors for CD, HLA DQ2 and/or HLADQ8, are present in about 40% of the Caucasian population. In addition, most of these individuals do not develop CD, since the prevalence of CD is ~1%. Another reason why avoiding gluten ingestion by a large part of the population is not desirable is that gluten-containing cereals (among others wheat, barley and rye) are important sources of dietary iron, fiber, calcium, folate, and vitamin B12 (12, 13). Much knowledge about the possible relationship between infant feeding practices and the development of CD has been obtained from "The Swedish epidemic of CD" during the mid1980s. Between 1985 and 1987, the incidence of CD in Swedish children younger than 2 years of age increased 4-fold, followed by a rapid decline in its incidence around 1995 (14). The occurrence of the epidemic was related to new dietary recommendations: delaying the introduction of all gluten-containing foods to infants until 6 months of age and changes in breastfeeding practices. In Sweden, the incidence of CD diminished when earlier introduction of gluten (>4 months) was reintroduced (14). Many retrospective studies have investigated this hypothesis that delayed introduction of gluten leads to CD with conflicting results. Results of observational studies suggested the existence of a "window of opportunity" for primary prevention, by introducing gluten between 4 and 6 months of age to reduce the risk of CD (Table 3). This and other early feeding practices, such as breastfeeding and breastfeeding at the time of gluten introduction, have been investigated as primary prevention strategies for reducing the risk of CD as well (Tables 4, 5). A systemic review and meta-analysis, which included all of the studies published on this topic between 1966 and 2004, found that breastfed children had a 52% reduction in the risk of being affected by CD compared to those who were not breastfed during the time of gluten introduction (pooled OR 0.48; 95% CI: 0.40 to 0.59) (37). However, all of these studies were observational and retrospective. Among the prospective studies that have been published, there are two gluten interventional ones, namely PREVENTCD and CELIPREV (15, 16) (Table 3):

- PREVENTCD is a multinational, randomized, double-blind, placebo-controlled dietary interventional study involving 944 children who had at least 1 first-degree relative with CD and HLA-DQ2 and/or DQ8. From age 4 to 6 months, 475 participants received 100 mg of vital gluten daily and 469 received placebo. After 24 weeks, intake of gluten was liberalized in both groups. CD serology was measured periodically. Children with elevated levels of CD antibodies and/or with symptoms suggestive of CD were offered small bowel biopsies to confirm the diagnosis. The results showed no significant difference between the groups receiving the early gluten intervention or placebo in the risk of developing CD at the age of 3 years.
- CELIPREV is an Italian multicenter, randomized, interventional study that compared early (at 6 months of age; n = 297) and delayed (at 12 months of age; n = 256) introduction of gluten into the diet of infants at risk for CD (first-degree relative with CD; HLA-DQ2 and/or DQ8 positivity). The results showed a reduced risk of developing CD by the age of 2 years in those with delayed introduction to gluten at 12 months, but no difference between groups in the risk of developing CD at 5 years of age. A few of the large, prospective, observational cohort (non-interventional) studies assessing the relationship between infant feeding practices and the risk of CD and/or CD autoimmunity (CDA) pointed out the following (Tables 3–5):
- The Generation R cohort study, including 1679 genetically susceptible CD children from the general population of Rotterdam, the Netherlands, showed that neither breastfeeding for 6 months or longer nor later exposure to gluten (>6 months) compared to earlier exposure (<6 months) was significantly associated with CDA (21)
- The Norwegian Mother and Child Cohort Study (MoBa) showed that breastfeeding longer than 12 months was associated with a higher risk of CD (22). However, this cohort only considered children with clinically diagnosed CD, so probably missed an important proportion of the children with CD.
- The BABYDIAB, a German cohort study, found no association between the duration of breastfeeding nor gluten introduction before or after 3 months of age and risk of CDA (26).
- The Environmental Determinants of Diabetes in the Young (TEDDY) project is an observational, prospective, cohort study that followed children at genetic risk for type 1 diabetes, wherein development of CD is a secondary outcome. The TEDDY study included 6,403 children with a genetic predisposition to developing CD in the United States, Finland, Germany, and Sweden. The study found that gluten introduction before 17 weeks of age or later than 26 weeks of age was not associated with an increased risk for CDA or CD; however, continuation of breastfeeding more than 1 month after gluten introduction compared with discontinuation of breastfeeding prior to gluten introduction was associated with increased risk of CDA but not of CD (20).

Table 3. Evidence of the effect of the timing of gluten introduction into the diet of young children and the risk of celiac disease

First author, year, study	Conclusion
Interventional studies	
Vriezinga 2014, PREVENTCD [15]	No significant difference in CD development at 3 years for gluten introduction at 4 months vs. 6 months^
Lionetti 2014, CELIPREV [16]	No significant difference in CD development at 5 years for gluten introduction at 6 months vs. 12 months
Sellitto 2012 [17]	No significant difference in CDA* risk for gluten introduction at 6 months vs. 12 months
Hummel 2011 [18]/ Beyerlein 2014[19]**	No significant difference in CD and CDA for different gluten introduction at 6 months vs. 12 months
Prospective cohort studies	
Aronsson 2015, TEDDY [20]	No significant difference in CD and CDA for gluten introduction at <17 vs. 17 – 26 vs. >26 weeks
Jansen 2014, Generation R [21]	No significant difference in CDA for gluten introduction at <6 months vs. > 6 months
Størdal 2013, MoBa [22]	Borderline significant difference in CD development at gluten introduction <6 months vs. >6 months
Welander 2010, ABIS [23]	No significant difference in CD for different times of gluten introduction from 0 to 12 months
Norris 2005, DAISY [24]	Significantly more CD with gluten introduction <3 or >7 months vs. gluten introduction between 4-6 months.
Ziegler 2003, BABYDIAB [25]	No significant difference in CD for gluten introduction ≤3 months vs > 6 months
Hummel 2007, BABYDIAB [26]	No significant difference in CDA for gluten introduction <3 months vs. >3 months
Retrospective studies	
Ivarsson 2002 [27]	Significantly more CD with gluten introduction > 6 months compared to gluten introduction between 4-6 months.
Peters 2001 [28]	No significant difference in CD gluten introduction at ≤3 vs >3 months
Falth-Magnusson 1996 [29]	No significant difference in CD for different times of gluten introduction
Cross-sectional study	
Ivarsson 2013, ETICS [30]	Significantly more CD with gluten introduction > 6 months compared to gluten introduction between 4-6 months.

CD: celiac disease; CDA: celiac disease autoimmunity
*= celiac disease autoimmunity
** = same population
^= months of age

Table 4. Most important studies on the evidence of protection from celiac disease with breastfeeding

First author, year, study	Conclusion
Interventional studies	
Vriezinga 2014, PREVENTCD [15]	No effect
Lionetti 2014, CELIPREV [16]	No effect
Prospective studies	
Jansen 2014, Generation R [21]	No effect
Størdal 2013, MoBa [22]	No effect*
Welander 2010, ABIS [23]	No effect
Norris 2005, DAISY study [24]	No effect
Ziegler 2003 [25]/Hummel 2007 [26]**, BABYDIAB	No effect
Retrospective studies	
Decker 2010 [31]	No effect
Roberts 2009 [32]	No effect
Ivarsson 2002 [27]	Protective
Peters 2001 [28]	Protective
Greco 1998 [33]	Protective
Ascher 1997 [34]	No effect
Falth-Magnusson 1996 [29]	Protective
Auricchio 1983 [35]	Protective
Cross-sectional study	
Ivarsson 2013, ETICS [30]	Protective

*=breastfeeding (BF)>1 year predisposing; **=same population

Table 5. Evidence of the effect of breastfeeding at the time of gluten introduction and risk for celiac disease

First author, year, study	Conclusion
Interventional studies	
Vriezinga 2014, PREVENTCD [15]	No effect
Lionetti 2014, CELIPREV [16]	No effect
Prospective cohort studies	
Aronsson 2016, TEDDY [36]	No effect
Størdal 2013, MoBa [22]	No effect
Hummel 2007, BABYDIAB [26]	No effect
Norris 2005, DAISY [24]	No effect
Retrospective studies	
Ivarsson 2002 [27]	Protective
Peters 2001 [28]	Protective
Ascher 1997 [34]	No effect
Falth-Magnusson1996 [29]	Protective

Two systematic reviews and meta-analyses, which included the above prospective interventional studies and large cohort studies (Tables 3–5), concluded that the timing of gluten introduction and the duration or maintenance of breastfeeding do not influence the development of CD (38, 39). Interest in the quantity of gluten at introduction into the diet of infants was also raised based on the results of the Swedish CD epidemic. The evaluation of results of one retrospective observational study indicated that large amounts of gluten (>16 g/day) at the time of first introduction increased the risk of CD (27). The same group of investigators further compared, in the ETIC project, 2 populations born in 1993 and 1997; they found a lower risk of CD in the population born in 1997 who ingested significantly less gluten-containing cereal compared to the population born in 1993 (24 vs. 38 g/day intake, respectively, under the age of 2 years) (30). Also, the Swedish case control study from the TEDDY cohort, in which gluten intake was assessed by dietary questionnaires, found that a high intake (>5.0 g/day) of gluten during the first 2 years of life was associated with an increased risk of CD (36). However, a similar analysis of the data in the international PREVENTCD study showed that the amount of gluten consumed at 11–36 months of age did not influence the risk for CD development (40). Thus, the influence of the amount of gluten intake on CD risk remains a topic of discussion. In accordance with the results from the above-mentioned studies, ESPGHAN has updated its guidelines for gluten introduction into the diet of young children. The current recommendation no longer suggests introducing gluten between 4 and 6 months of age; rather they recommend that gluten may be introduced into the infant's diet anytime between 4 and 12 completed months of age, since gluten introduction in these infants does not seem to influence the absolute risk of developing CDA or CD during childhood (38). In addition to gluten and breastfeeding, other environmental factors may be involved in the risk and/or prevention of CD. Identifying and influencing these factors may lead to preventive strategies. Some of these factors are discussed below.

(Intestinal) infections

Intestinal infections might change gut permeability and lead to the passage of immunogenic gluten peptides through the epithelial barrier, and thus, activate an autoimmune reaction. Many groups have studied the relationship between infections, both viral and bacterial, and the risk of CD, with varying results (Table 6). The role of early infections was retrospectively explored in the Swedish population-based incident case referent ETICS study. Having three or more parental-reported infections, regardless of the type of infection, during the first 6 months of life was associated with significantly increased risk of CD, even after adjusting for infant feeding and socioeconomic status (61). Results of prospective studies are contradictory. Data from the PREVENTCD study showed no significant difference in the cumulative incidence of CD between children with and without parental-reported gastrointestinal infections in the first 18 months of life (15).

Table 6. Some of the most relevant studies[#] on infections and the risk of celiac disease or celiac disease autoimmunity

First author, year, study	Pathogen	Association between CD(A)
Prospective studies		
Stene 2006 [41]	Rotavirus	Positive
Thevenot 2007 [42]	Hepatitis C virus	None
Gravina 2012 [43]	Hepatitis C virus	None
Jansen 2016 [44]	EBV, CMV and HSV-1	Negative
Karhus 2018 [45]	Influenza	None
Dore 2018 [46]	Helicobacter Pylori	None
Retrospective studies		
Lahdeaho 1993 [47]	Adenovirus 12/40	Positive
Vesy 1993 [48]	Adenovirus 12, CMV, HSV	None
Kagnoff 1987 [49]	Adenovirus 12 Adenovirus 18/echovirus 11	Positive None
Mahon 1991 [50]	Adenovirus 12	None
Fine 2001 [51]	Hepatitis C virus	Positive
Carlsson 2002 [52]	Enterovirus	None*
Villalta 2005 [53]	Hepatitis C virus	Positive
Ruggeri 2008 [54]	Hepatitis C virus	Positive
Sarmiento 2012 [55]	Enterovirus, EBV, CMV, Hepatitis C virus	Positive
Tjernberg 2014 [56]	RSV	Positive
Abid 2016 [57]	Hepatitis B virus	Positive
Tarish 2016 [58]	Adenovirus	None
Alaedini 2017 [59]	Borrelia	None
Bouziat 2017 [60]	Reovirus	Positive

CD: celiac disease; CDA: celiac disease autoimmunity; EBV: Epstein Barr virus; CMV: cytomegalovirus; HSV: herpes simplex virus; RSV: respiratory syncytial virus.

[#]= case reports were excluded

***=** between these infection during pregnancy and CD development in the offspring

However, the TEDDY study found that parental-reported early gastrointestinal infections increased the risk of CDA within the following 3 months (HR 1.33; 95% CI 1.11–1.59). This effect was observed particularly in those children with non-HLA-DQ2 genotypes who had been breastfed for been breastfed for < 4 months, as well as in children born in winter and introduced to gluten before the age of 6 months (62). In the prospective MoBa study, children with ≥10 infections (respiratory and gastrointestinal) before 18 months of age had a higher risk of being clinically diagnosed with CD compared with children who had ≤4 infections, even after adjustments for antibiotic exposure (63). Viral infections have been suggested to play a role in the development of CD (Table 6), and recently, reovirus has been reported as a trigger for the disease, both in vitro as well as in vivo (60). In vitro, reovirus infection induced a disruption of intestinal immune homeostasis and initiated

loss of oral tolerance and T-helper inflammatory immunity to dietary antigens. In CD patients anti-Reovirus antibodies were significantly overrepresented in comparison to health controls. However, this disruption of the immune homeostasis may not be exclusive to reovirus and their role in the development of CD should be studied prospectively.

Type of delivery

The mode of delivery (vaginal or cesarean section [C-section]) has a strong influence on shaping the initial gut microbiota composition. It has been hypothesized that infants born by C-section acquire different bacterial communities compared to vaginally delivered infants (64), which may influence the short- and long-term immune responses to environmental factors, thereby predisposing to autoimmunity (65). Also, the type of C-section, emergency vs. elective, has been hypothesized as a different possible influencing factor, since the cord blood immune cell phenotypes are affected by stress during vaginal delivery and this does not happen by elective C-section (66). In addition, infants born vaginally and during emergency C-section are colonized at first by fecal and vaginal bacteria of the mother, whereas infants born through elective cesarean delivery are exposed initially to bacteria originating from the hospital environment and healthcare workers. Infants born by cesarean delivery are characterized by a more slowly diversifying microbiota, with a substantial absence of Bifidobacteria species and Bacteroides and the presence of facultative anaerobes, such as Clostridium species. These differences might influence the development of the mucosal immune system, the establishment of a stable intestinal host-microbial homeostasis, as well as the mucosal barrier function and ultimately contribute to the risk of acquiring immune-mediated diseases, such as CD, later in life (67).

Some studies have identified C-section as a risk factor for the development of CD (68, 69). However, more recent prospective studies have found no association (70–73) (Table 7). Recently, a large, observational, register-based, cohort study investigated the association between the type of delivery and the risk of developing CD in two independent population cohorts (Denmark, birth cohort 1995–2010 and Norway, birth cohort 2004–2012) (74). A total of 3,314 children were diagnosed with CD. C-sections were performed in 286,640 children, and the mode of delivery was not associated with an increased risk of diagnosed CD.

In the above-mentioned Danish cohort, the association between elective C-section and diagnosed CD was positive and reached borderline statistical significance after adjusting for year of birth, sex, maternal age, education, parity, gestational age, and weight for gestational age (OR: 1.20; 95% CI: 1.00–1.43).

However, this finding was not replicated in the corresponding Norwegian cohort (OR: 0.96; 95% CI: 0.79–1.17) (74). Analysis of the data from the Swedish Medical Birth Register between 1973 and 2008, comparing cases with villous atrophy with age- and sex-matched controls from the general population, found a weak association between an elective C-section and CD in offspring (adjusted odds ratio [OR] = 1.15), but no increased risk for CD diagnoses after an emergency (adjusted OR = 1.02) or any C-section (adjusted OR = 1.06) (69). Data from a population- and national register-based cohort including all children born in Denmark from January 1997 to December 2012 showed the opposite: children delivered by emergency C-section were at an increased risk for CD (adjusted OR = 1.22), whereas children delivered by elective C-section were not (adjusted OR = 0.69) (68). Thus, despite the plausible hypothesis that mode of delivery affects risk of CD, the current literature showed no association between the type of delivery and the risk of CD (Table 7).

Table 7. Some of the most relevant studies on type of delivery and the risk for celiac disease

First author, year, study	Conclusion
Koletzko 2018, TEDDY [73]	No association with CDA or CD
Dydensborg Sander 2018, ETICS [74]	No association with CD
Lionetti 2017, CELIPREV [72]	No association with CD
Kristensen, 2016 [68]	Positive association between emergency CS and CD
Emilsson 2015, MoBa [70]	No association between CS and CD
Sevelsted 2015 [71]	No association with CD
Marild 2012 [69]	Positive association with CD
Decker 2010 [31]	Positive association with CD
Roberts 2009 [32]	Negative association between CS and CD

CD: celiac disease; CDA: celiac disease autoimmunity; CS: caesarean section

Antibiotics

The ETIC study found no evidence of increased CD risk with antibiotic use in the first 6 months of life (61). However, other 2 retrospective studies have shown a positive association between antibiotic use and CD risk (75, 76). A recent analysis of the TEDDY study showed that cumulative exposure to β-lactam or macrolide antibiotics, up to 6 months, during the first or second year of life and within 6 months before the seroconversion period, was not associated with CDA. Also, maternal use of antibiotics during pregnancy was also evaluated as a risk factor and did not significantly contribute to CDA risk in this study. In conclusion, the role of antibiotics in the development of CD is a topic that remains unclear and requires more research.

Microbiota

CD development has also been linked to alterations in the human gut microbiome, which is necessary for proper development of the immune system and establishment of oral tolerance in early life (65). The contributing role of perturbations in the gut microbiota, and of specific enteric bacteria, to gluten-induced immunopathology has been shown in animal models (77). PROFICEL, a prospective study of 164 healthy Spanish newborns with a first-degree relative with CD and HLA-DQ2 and/or DQ8 positivity, reported an association between the HLA-DQ genotype and the intestinal microbiota composition. In this study, the HLA-DQ2/8 genotype and the type of feeding (breastfeeding or formula) were shown to influence, in conjunction, the composition of the intestinal microbiota (78). The high-risk genotype for developing CD (HLA-DQ2, including homozygous HLA-DQ2.5 or heterozygous DQ2.5/DQ2.2 and DQ2.2/DQ7.5) was associated with reduced numbers of Bifidobacterium, specifically of the species B. Longum, compared to the rest of the lower-risk genotypes (79). Also, other studies have found similar results; the duodenal and fecal microbiota of CD patients is unbalanced, with decreased numbers of anti-inflammatory bacteria, such as Bifidobacterium spp. and increased numbers of Bacteroides spp., which are only partially normalized after a long-term gluten free diet (GFD) (80–82). In a double-blind, randomized, placebo-controlled, interventional trial performed in children with newly diagnosed CD, children were randomized to receive Bifidobacterium longum or placebo in conjunction with a GFD (83). A decrease in both the numbers of the Bacteroides fragilis group and the fecal secretory IgA concentration was found, which might further confirm the role of microbiota in the pathogenesis of CD. But, so far, studies have failed to find a distinct microbiota profile in patients with CD. A sub-study of the PROFICEL project, including 10 CD cases and 10 matched controls, suggests altered early proportions of Firmicutes and members of the Actinobacteria phylum (B. Longum) in children who later progressed to CD (84). Hopefully, the results of the Celiac Disease Genomic, Environmental, Microbiome, and Metabolomic (CDGEMM) study, a multicenter, longitudinal study of infants at risk for CD, will provide an answer to the question regarding the role of the gut microbiome and the risk of CD (85). CDGEMM aims to enroll 500 infants aged 0–6 months with a first-degree family member with CD. Health status, anthropometrics, nutritional information, household and environmental information, and blood and stool samples are being collected regularly to understand the role of the gut microbiome as an additional factor that may play a key role in early steps involved in the development of autoimmune disease (85). In conclusion, in the field of primary prevention, infant feeding practices have been explored by interventional studies with long-term follow up, but have shown no protection for risk of CD. Other possible influences on the development of CD, especially the role of infections and the gut microbiome, need further research.

Text box

Summary of evidence of effectiveness of possible primary prevention strategies for celiac disease	Conclusion
Infant feeding	
Breastfeeding	No effect
Breastfeeding at the time of gluten introduction	No effect
Timing of gluten introduction	No effect
Amount of gluten at the time of gluten introduction	Unclear
(Intestinal) infections	Unclear
Type of delivery	No effect
Antibiotics	Unclear
Microbiota	Unknown

SECONDARY PREVENTION

Case finding

Secondary prevention focuses on early detection and treatment (Table 8). Active case finding refers to the liberal diagnostic testing of subjects with CD-associated symptoms. In the general population, this approach has led to the early diagnosis of many patients, resulting in significant health improvement after treatment, good compliance with the GFD, and good CD-related QoL (86, 87); unfortunately, however, it does not counter the entire under-diagnosis of CD (88, 89). Only a small proportion of the undiagnosed patients are detected with this strategy, since ~50% of the children in screening-detected studies have symptoms at the time of diagnosis (15, 16, 90).

Table 8. Secondary prevention strategies for celiac disease

Case finding
Screening in high-risk groups
First-degree relatives of CD patients
Type 1 diabetes mellitus
Autoimmune thyroid disease
Autoimmune liver disease
Syndrome: Down, Turner, Williams
IgA deficiency
Mass screening

CD: celiac disease

Screening for celiac disease in high-risk groups

Because of the high prevalence of CD among these groups, evidence-based guidelines recommend screening for early detection of the disease (7) (Table 8). A plethora of studies are available on 2 of the populations who belong to these high-risk groups, namely first-degree relative of patients with CD and children with type 1 diabetes mellitus (T1DM).

First-degree relatives of CD patients

Many studies have demonstrated that first-degree relatives (FDRs) of celiac patients have a higher risk of developing CD than the general population, with a prevalence ranging from 1.6 to 38% (91). Based on a systematic review and meta-analysis, Sing et al. (91) reported that the pooled prevalence of CD was 7.5% in 10,252 FDRs (91). The risk of developing CD among FDRs is influenced by gender and HLA haplotype (15, 16). CD occurs more often in girls (female: male ratio of 2–3:1), and HLADQ2 homozygous children have a significantly higher risk of developing CD than HLADQ2 heterozygous children (14.9 vs. 3.9%, respectively, at the age of 3 years) (15).

Children with conditions/diseases associated with CD

The prevalence of CD in patients with T1DM has been reported by most studies as ranging between 4 and 10% (92). Many children with T1DM and CD are asymptomatic or at least symptoms of CD have not been observed. In these cases, CD may only be detected by serologic screening. However, it has been shown that strict adherence to a GFD was < 30% in children with both CD and T1DM, compared to 81% among patients with CD only (93). Maintaining a strict GFD in addition to a diabetic diet requires additional time, effort, and expense. Evidence is inconclusive as to whether the benefits of screening and potentially treating asymptomatic individuals outweigh the harms of managing a population already burdened with a serious illness. The Celiac Disease and Diabetes-Dietary Intervention and Evaluation Trial (CD-DIET) (ClinicalTrials.gov Identifier: NCT01566110) involves screening of children and adults with T1DM for asymptomatic CD, followed by randomization to a GFD or no-GFD group, to assess outcomes (including diabetes control, bone mineral density, and HRQoL) over 1 year to clarify effects of screening and treating asymptomatic CD in this population with a GFD (94).

Mass screening

Screening the general population, also called mass screening, would theoretically be the best form of secondary prevention since it could potentially detect all cases of CD, including those in asymptomatic patients as well as those in patients who lack symptoms. Results from most screening studies performed in the general population suggest that symptoms are not reliable predictors of CD (15, 95, 96), reinforcing the place of mass screening as the best strategy for secondary prevention of CD enabling early treatment to reduce the burden of morbidity and mortality associated with untreated CD (97, 98). However, mass screening for CD is still debated, partly because evidence has been lacking on the accuracy of diagnostic tests and on the health benefits after diagnosis and treatment of asymptomatic detected patients. This uncertainty also affects the cost benefit of mass screening, which is needed for implementation of mass screening for CD (99, 100). Most studies on the diagnostic accuracy of diagnostic tests

for CD have been conducted in symptomatic patients (101, 102). Because the positive predictive value declines when the test is used in settings with a low pre-test prevalence, such as the general population, the sensitivity and specificity of these tests are lower in the setting of mass screening. Recently, a prospective study performed in Rotterdam has shown a positive predictive value of 81% for CD in the general pediatric population (96). These authors also showed that undiagnosed CD is associated with a lower body mass index compared to controls at the age of 9 years (96) and associated with fetal growth restriction and lower birth and placental weight during pregnancy (8). Additional information about the importance and effectiveness of screening comes from a population-based-screening study performed in Sweden; this study showed that at 10 years of age, children with CD detected by screening already had reduced bone mineral density in the total body and spine compared with age-matched controls. These differences were not found in children with CD on a GFD from 3 years of age, indicating that children with screening-detected CD benefit from early diagnosis and treatment (103). The data on the benefits and harms of screening are limited. Only one randomized trial evaluated the effectiveness of GFD vs. no GFD in apparently asymptomatic adults with screen-detected CD and found that initiation of a GFD in screen-detected adults with unrecognized symptoms was associated with improved gastrointestinal symptoms (104). Other traditional reason against mass screening is that adherence to the GFD in minimally or asymptomatic patients would be lower than in symptomatic patients and that the QoL is decreased in screening-detected CD patients following a GFD. However, 10 years of follow up among Dutch children and the results of a sub-study from the ETICS project showed similar adherence rates to the GFD in screening detected children compared with clinically detected children (105, 106). No significant differences in HRQoL were observed between screening-detected and symptom-detected adult patients (107, 108). Furthermore, a systematic review and meta-analysis on dietary adherence and HRQoL in adult patients with CD detected by screening showed a significantly lower HRQoL after 1 year of treatment with a GFD in symptom-detected patients compared to screening detected patients (109). Despite the aforementioned literature that is positive about screening of the general population, the current literature recommending mass screening is limited.

TERTIARY PREVENTION

Gluten-free diet (GFD)

Tertiary prevention focuses on reducing the impact of existing disease by improved treatment (Table 9). One of these strategies involves optimizing adherence to the GFD. Complete removal of gluten from the diet is a challenge, as gluten is present in a wide

variety of foods. However, since the introduction of allergen labelling in the European Union (EU) in 2005, gluten cannot be hidden in products. The amount of gluten capable of initiating an antigenic reaction has been estimated to be >20 mg/kg (or parts per million = ppm) of gluten, and contamination below 20 ppm is considered safe over a wide range of foods in daily consumption.

Table 9. Tertiary prevention strategies for celiac disease

Strategy	Successful
Optimal adherence to the GFD	Yes
Treatment options for CD other than a GFD	
Larazotide acetate	Unclear
Endopeptidases	
Latiglutenase (ALV003)	Unclear
Aspergillus niger prolyl endoprotease (AN-PEP)	Unclear
Desensitization therapy (therapeutic vaccine)	Unknown

CD: celiac disease; GFD: gluten-free diet

Improving monitoring of and adherence to the GFD

Dietitian

Due to the complexity of the GFD, it is essential that newly diagnosed patients be referred to a dietitian with expertise in CD. A delay in referral, or no referral at all, increases the likelihood of the patient obtaining inaccurate information from the Internet, health food stores, alternative health practitioners, family, friends, and other sources, often resulting in confusion, frustration, and insufficient knowledge regarding CD and the GFD (110). Gluten-containing cereals, such as wheat, barley, and rye, are important sources of dietary iron, calcium, folate, and vitamin B12. As the treatment of CD with a GFD can lead to nutritional deficiencies, the support of a dietitian is necessary to avoid these deficiencies. Also, consultation with someone with knowledge in the field of replacement (gluten-free) products, such as amaranth, buckwheat, quinoa, sorghum and teff, is of great importance and could improve intakes of protein, iron, calcium, and fibre by patients with CD (111).

Validated food questionnaires

A dietary interview to assess compliance with the GFD is the best way to detect errors in GFD adherences among children and young adults, but it is time-consuming (20–30 min per patient) and requires expert personnel. Several short questionnaires have been developed to measure GFD adherence in order to save time, and while some are not sensitive enough, others are useful in assessing compliance to the diet (112). With the increasing use of self-assessment and alternative follow-up methods for CD patients, including electronic patient records and E-health tolls, completing questionnaires be-

fore or during a medical consultation should be easily implemented in the healthcare of children and young adults with CD (113).

Measurement of gluten immunogenic peptides (GIPs)

Available methods to assess GFD compliance are time-consuming and are also insufficiently sensitive to detect occasional dietary transgressions that may cause gut mucosal damage. Determination of serum TG2A is usually used during the follow-up of a patient on a GFD, as this marker improves with gluten elimination (114). However, it has been reported that even while following a GFD, children and women with CD have a much higher prevalence of gastrointestinal symptoms than controls, and they also use healthcare services more often (115). As mucosal damage may still persist without TG2A, antibody testing may be negative in patients with only partial adherence to the GFD (116). Therefore, it is necessary to have a non-invasive biomarker to monitor compliance with the GFD. Certain GIPs are resistant to gastrointestinal digestion and can interact with the immune system of patients with CD to trigger an autoimmune response against transglutaminase and other antigens. A proportional fraction of the GIPs absorbed in the gastrointestinal tract make it to the circulation and are excreted in urine. GIPs are detectable in concentrated urines and may be useful in clinical practice as a monitoring tool to follow-up compliance with the GFD. GIPs are detected in urine samples 6–48 h after gluten intake (>25 mg) and remained detectable for 1–2 days (117).

Treatment options for CD other than the GFD

Several other treatments aimed at different pathogenic targets of CD have been studied in recent years: modification of gluten to produce non-immunogenic gluten, endoluminal therapies to degrade gluten in the intestinal lumen, increasing gluten tolerance, modulation of intestinal permeability, and regulation of the adaptive immune response. However, not all of these therapies have been tested in clinical trials (yet). The most advanced studies are devoted to larazotide acetate and prolyl-endopeptidases degrading toxic gluten peptides and to therapeutic vaccination.

Larazotide acetate

Patients with active CD have increased intestinal permeability. Zonulin, a modulator of epithelial tight junctions, is overexpressed in these patients. Release of zonulin in response to binding between gliadin peptides and a specific chemokine receptor (CXCR3) results in a measurable reduction in the usual intestinal barrier and allows enhanced passage of gliadin. This mechanism has been the target of advanced research that led to the development of larazotide acetate (AT-1001), an octapeptide that inhibits gliadin-induced intestinal permeability. Several phase I and II clinical trials have confirmed the safety of this agent and suggest a potential beneficial effect of larazotide (118,

119). Additionally, patients who were treated with larazotide acetate had significantly fewer symptoms (patient reported Celiac Disease Gastrointestinal System Rating Score) compared with those taking a placebo (120–122). A dose-response effect was not seen, with the most benefit encountered at the lowest (0.5 mg) of 4 dosages administered (121); however, this study did not measure histologic endpoints, and larazotide had no significant effect on serologic levels of specific CD antibodies as TG2A.

Endopeptidases

The gluten peptides, which are responsible for inducing the immunological response in CD patients, are rich in proline and are highly resistant to enzymatic proteolysis within the digestive tract. For many years, there have been studies conducted to investigate the effectiveness of orally administered prolyl oligopeptidases in the degradation of toxic gliadin peptides before they reach the mucosa of the small intestine. Latiglutenase (ALV003, Alvine Pharmaceuticals, San Carlos, CA, USA) is an orally administered mixture of 2 recombinant gluten-specific proteases—a cysteine protease (EP-B2) and a prolyl endopeptidase (PEP)—which have been shown in vitro to degrade gluten (123). Both endopeptidases are active and stable at gastric pH (124). In a Phase 2 study with ALV003, adults with biopsy-proven CD were randomly assigned to groups receiving ALV003 or placebo, together with a daily 2 g gluten challenge. Upper endoscopy was performed at baseline and after the gluten challenge. Primary endpoint included the villus height to crypt depth ratio and CD3+ intra-epithelial lymphocytes (IEL) density. Serologic markers and symptoms were also assessed. In the ALV003 group, there were no changes in histological measures, while in the placebo group, evidence of mucosal injury was shown after gluten challenge. In contrast, no differences were seen in symptoms and serologic markers of CD in both groups. In a phase 2 study involving patients with symptomatic CD and histologic evidence of significant duodenal mucosal injury, ALV003 did not improve histologic and symptom scores when compared with placebo (125). However, a subgroup-analysis of the study showed a statistically significant, dose-dependent reduction in the severity and frequency of symptoms in seropositive but not in seronegative patients (126).

Aspergillus niger prolyl endoprotease (AN-PEP; DSM, Heerlen, The Netherlands) is also an endopeptidase, isolated from the fungus Aspergillus niger. The enzyme is active between a pH of 2 and 8, with an optimum activity at pH 4–5, thus, in the stomach and small intestine (127). In a randomized, placebo-controlled, crossover study, 18 self-reported gluten-sensitive subjects consumed a porridge containing 0.5 g gluten together with two tablets containing either a high or low dose of ANPEP or placebo. Gastric and duodenal contents were sampled over 180 min. The primary outcome was defined as the efficacy of the high dose of AN-PEP compared with placebo in degrading at least 50% of gluten,

based on the amount of gluten detected in the duodenum. The researchers concluded that the AN-PEP enzyme is effective in degrading small amounts of gluten as part of a complex meal in the stomach, but it is not intended to replace a GFD in patients with gluten-related disease (128). In a Phase 2a double-blinded, placebo-controlled, randomized trial, 16 CD patients on a GFD, who were in serological and histopathological clinical remission, were administered AN-PEP or a placebo with gluten-containing toast (~7 g/day gluten). The mean score for the gastrointestinal subcategory of the CD quality (CDQ) was relatively high throughout the study, indicating that AN-PEP was well-tolerated. In the efficacy phase, the CDQ scores of patients consuming gluten with placebo or gluten with AN-PEP did not significantly deteriorate and, moreover, no differences between the groups were observed. Larazotide and PEPs will not become an alternative to the GFD and their potential role as therapeutic agents for CD remain unclear.

Desensitization therapy (therapeutic vaccine)

NexVax2 from ImmusanT is a desensitizing vaccine that uses three dominant gluten peptides administered subcutaneously to induce an immune response in CD patients who carry the immune recognition gene HLA-DQ2.5, which accounts for disease in 80–90% of patients. The aim of the vaccine is to use peptide-based immunotherapy to shift the Tcell response from pro-inflammatory to regulatory, in order to restore immune tolerance to gluten. Phase 1b clinical trials of this vaccine have recently been completed supporting the safety, tolerability and relevant bioactivity of Nexvax2 (129).

CONCLUSIONS

- Celiac disease is a common autoimmune disorder induced by ingestion of gluten in genetically susceptible individuals.
- Only a minority of those who are at genetic risk develop the disease.
- The incidence of CD has increased over the last half-century, resulting in rising interest in identifying risk factors for CD to enable prevention.
- Environmental and/or lifestyle factors play a causal role in the development of CD.
- For primary prevention (i.e., interventions before CD occurs), early feeding practices seem to have no impact on the risk of developing CD during childhood. Other environmental influences have been investigated as potential risk factors; however, they have not yet led to primary prevention strategies.
- Secondary prevention is possible through early diagnosis and treatment; however, it will not identify all CD patients as long as mass screening has not been introduced.

- As a gluten-free diet is a major challenge, tertiary prevention strategies are under evaluation; however, none of these measures are currently recommended as treatment.

REFERENCES

1. Ludvigsson JF, Rubio-Tapia A, Van Dyke CT, Melton LJ III, Zinsmeister AR, Lahr BD, et al. Increasing incidence of celiac disease in a North American population. Am J Gastroenterol. (2013) 108:818–24. doi: 10.1038/ajg.2013.60
2. Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, et al. Increasing prevalence of celiac disease over time. Aliment Pharmacol Ther. (2007) 26:1217–25. doi: 10.1111/j.1365-2036.2007.03502.x
3. Vavricka SR, Vadasz N, Stotz M, Lehmann R, Studerus D, Greuter T, et al. Celiac disease diagnosis still significantly delayed—doctor's but not patients' delay responsive for the increased total delay in women. Dig Liver Dis. (2016) 48:1148–54. doi: 10.1016/j.dld.2016.06.016
4. Csizmadia CGDA, Mearin ML, von Blomberg BM, Brand R, VerlooveVanhorick SP. An iceberg of childhood celiac disease in the Netherlands. Lancet (1999) 353:813–4. doi: 10.1016/S0140-6736(99)00243-3
5. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: am involving spectrum. Gastroenterology (2001) 120:636–51. doi: 10.1053/gast.2001.22123
6. Mearin ML. Celiac disease among children and adolescents. Curr Probl Pediatr Adolesc Health Care (2007) 37:86–105. doi: 10.1016/j.cppeds.2007.01.001
7. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr. (2012) 54:136–60. doi: 10.1097/MPG.0b013e31821a23d0
8. Kiefte-de Jong JC, Jaddoe VW, Uitterlinden AG, Steegers EA, Willemsen SP, Hofman A, et al. Levels of antibodies against tissue transglutaminase during pregnancy are associated with reduced fetal weight and birth weight. Gastroenterology (2013) 144:726–35. doi: 10.1053/j.gastro.2013.01.003
9. Green PHR, Jabri B. Celiac disease. Lancet (2003) 362:383–91. doi: 10.1016/S0140-6736(03)14027-5
10. Mearin ML. The prevention of celiac disease. Best Pract Res Clin Gastroenterol. (2015) 29:493–501. doi: 10.1016/j.bpg.2015.04.003
11. Maars van der PJ, Mackenbach JP. Volksgezondheid en Gezondheidszorg. Elsevier; Bunge (1999). Tweede druk [Dutch].
12. Hopman EG, le Cessie S, von Blomberg BM, Mearin ML. Nutritional management of the gluten-free diet in young people with celiac disease in The Netherlands. J Pediatr Gastroenterol Nutr. (2006) 43:102–8. doi: 10.1097/01.mpg.0000228102.89454.eb
13. Ohlund K, Olsson C, Hernell O, Ohlund I. Dietary shortcomings in children on a gluten-free diet. J Hum Nutr Diet. (2010) 23:294–300. doi: 10.1111/j.1365-277X.2010.01060.x
14. Ivarsson A, Persson LA, Nyström L, Ascher H, Cavell B, Danielsson L, et al. Epidemic of celiac disease in Swedish children. Acta Pediatr. (2000) 89:165–71. doi: 10.1111/j.1651-2227.2000.tb01210.x
15. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med. (2014) 371:1304–15. doi: 10.1056/NEJMoa1404172
16. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med. (2014) 371:1295–303. doi: 10.1056/NEJMoa1400 697

17. Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, Gajer P, et al. (2012). Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. PLoS ONE 7:e33387. doi: 10.1371/journal.pone.0033387
18. Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care (2011) 34:1301–5. doi: 10.2337/dc10-2456
19. Beyerlein A, Chmiel R, Hummel S, Winkler C, Bonifacio E, Ziegler AG. Timing of gluten introduction and islet autoimmunity in young children: updated results from the BABYDIET study. Diabetes Care (2014) 37:e194–5. doi: 10.2337/dc14-1208
20. Andrén Aronsson CA, Lee HS, Liu E, Uusitalo U, Hummel S, Yang J, et al. Age at gluten introduction and risk of celiac disease. Pediatrics (2015) 135:239–45. doi: 10.1542/peds.2014-1787
21. Jansen MA, Tromp II, Kiefte-de Jong JC, Jaddoe VW, Hofman A, Escher JC, et al. Infant feeding and anti-tissue transglutaminase antibody concentrations in the Generation R Study. Am J Clin Nutr. (2014) 100:1095– 101. doi: 10.3945/ajcn.114.090316
22. Størdal K, White RA, Eggesbo M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatric (2013) 132:1202-9. doi: 10.1542/peds.2013-1752
23. Welander A, Tjernberg AR, Montgomery SM, Ludvigsson J, Ludvigsson JF. Infectious disease and risk of later celiac disease in childhood. Pediatrics (2010) 125:e530–e536. doi: 10.1542/peds.2009-1200
24. Norris JM, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE, et al. Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. JAMA (2005) 293:2343–51. doi: 10.1001/jama.293.19.2343
25. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E, et al. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA (2003) 290:1721–8. doi: 10.1001/jama.290.13.1721
26. Hummel S, Hummel M, Banholzer J, Hanak D, Mollenhauer U, Bonifacio E, et al. Development of autoimmunity to transglutaminase C in children of patients with type 1 diabetes: relationship to islet autoantibodies and infant feeding. Diabetologia (2007) 50:390–4. doi: 10.1007/s00125-006-0546-3
27. Ivarsson A, Hernell O, Stenlund H, Persson LA. Breast-feeding protects against celiac disease. Am J Clin Nutr. (2002) 75:914–21. doi: 10.1093/ajcn/75.5.914
28. Peters U, Schneeweiss S, Trautwein EA, Erbersdobler HF. A case-control study of the effect of infant feeding on celiac disease. Ann Nutr Metab. (2001) 45:135–42. doi: 10.1159/000046720
29. Falth-Magnusson K, Franzen L, Jansson G, Laurin P, Stenhammar L. Infant feeding history shows distinct differences between Swedish celiac and reference children. Pediatr Allergy Immunol. (1996) 7:1–5. doi: 10.1111/j.1399-3038.1996.tb00098.x
30. Ivarsson A, Myléus A, Norström F, van der Pals M, Rosén A, Högberg L, et al. Prevalence of childhood celiac disease and changes in infant feeding. Pediatrics (2013) 131:e687–94. doi: 10.1542/peds.2012-1015
31. Decker E, Engelmann G, Findeisen A, Gerner P, Laass M, Ney D, et al. Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics (2010) 125:e1433–40. doi: 10.1542/peds.2009-2260
32. Roberts SE, Williams JG, Meddings D, Davidson R, Goldacre MJ. Perinatal risk factors and celiac disease in children and young adults: a record linkage study. Aliment Pharmacol Ther. (2009) 29:222–31. doi: 10.1111/j.1365-2036.2008.03871.x

33. Greco L, Auricchio S, Mayer M, Grimaldi M. Case control study on nutritional risk factors in celiac disease. J Pediatr Gastroenterol Nutr. (1988) 7:395–9. doi: 10.1097/00005176-198805000-00013
34. Ascher H, Krantz I, Rydberg L, Nordin P, Kristiansson B. Influence of infant feeding and gluten intake on celiac disease. Arch Dis Child. (1997) 76:113–7. doi: 10.1136/adc.76.2.113
35. Auricchio S, Follo D, de Ritis G, Giunta A, Marzorati D, Prampolini L, et al. Does breast feeding protect against the development of clinical symptoms of celiac disease in children? J Pediatr Gastroenterol Nutr. (1983) 2:428–33. doi: 10.1097/00005176-198302030-00006
36. Andrén Aronsson C, Lee HS, Koletzko S, Uusitalo U, Yang J, Virtanen SM, et al. TEDDY Study Group. Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish Birth Cohort. Clin Gastroenterol Hepatol. (2016) 14:403–9. doi: 10.1016/j.cgh.2015.09.030
37. Akobeng AK, Ramanan AV, Buchan I, Heller RF. Effect of breast feeding on risk of celiac disease: a systematic review and meta-analysis of observational studies. Arch Dis Child. (2006) 91:39–43. doi: 10.1136/adc.2005.082016
38. Szajewska H, Shamir R, Chmielewska A, Pie´scik-Lech M, Auricchio R, Ivarsson A, et al. PREVENTCD Study Group. Systematic review with metaanalysis: early infant feeding and celiac disease–update 2015. Aliment Pharmacol Ther. (2015) 41:1038–54. doi: 10.1111/apt.13163
39. Silano M, Agostoni C, Sanz Y, Guandalini S. Infant feeding and risk of developing celiac disease: a systematic review. BMJ Open (2016) 6:e009163. doi: 10.1136/bmjopen-2015-009163
40. Crespo-Escobar P, Mearin ML, Hervás D, Auricchio R, Castillejo G, Gyimesi J, et al. The role of gluten consumption at an early age in celiac disease development: a further analysis of the prospective PreventCD cohort study. Am J Clin Nutr. (2017) 105:890–6. doi: 10.3945/ajcn.116.144352
41. Stene LC, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. (2006) 101:2333– 40. doi: 10.1111/j.1572-0241.2006.00741.x
42. Thevenot T, Denis J, Jouannaud V, Monnet E, Renou C, Labadie H, et al. Celiac disease in chronic hepatitis C: a French multicentre prospective study. Aliment Pharmacol Ther. (2007) 26:1209–16. doi: 10.1111/j.1365-2036.2007.03499.
43. Gravina AG, Federico A, Masarone M, Cuomo A, Tuccillo C, Loguercio C, et al. Celiac disease and C virus-related chronic hepatitis: a non association. BMC Res Notes (2012) 5:533. doi: 10.1186/1756-0500-5-533
44. Jansen MA, van den Heuvel D, van der Zwet KV, Jaddoe VW, Hofman A, Escher JC, et al. Herpesvirus infections and transglutaminase type 2 antibody positivity in childhood: the Generation R Study. J Pediatr Gastroenterol Nutr. (2016) 63:423–30. doi: 10.1097/MPG.0000000000001163
45. Kårhus LL, Gunnes N, Størdal K, Bakken IJ, Tapia G, Stene LC, et al. Influenza and risk of later celiac disease: a cohort study of 2.6 million people. Scand J Gastroenterol. (2018) 53:15–23. doi: 10.1080/00365521.2017.1362464
46. Dore MP, Salis R, Loria MF, Villanacci V, Bassotti G, Pes GM. Helicobacter pylori infection and occurrence of celiac disease in subjects HLADQ2/DQ8 positive: a prospective study. Helicobacter (2018) 23:e12465. doi: 10.1111/hel.12465
47. Lähdeaho ML, Parkkonen P, Reunala T, Mäki M, Lehtinen M. Antibodies to E1b protein-derived peptides of enteric adenovirus type 40 are associated with celiac disease and dermatitis herpetiformis. Clin Immunol Immunopathol. (1993) 69:300–5. doi: 10.1006/clin.1993. 1184
48. Vesy CJ, Greenson JK, Papp AC, Snyder PJ, Qualman SJ, Prior TW. Evaluation of celiac disease biopsies for adenovirus 12 DNA using a multiplex polymerase chain reaction. Mod Pathol. (1993) 6:61–4.

49. Kagnoff MF, Paterson YJ, Kumar PJ, Kasarda DD, Carbone FR, Unsworth DJ, et al. Evidence for the role of a human intestinal adenovirus in the pathogenesis of celiac disease. Gut. (1987) 28:995–1001. doi: 10.1136/gut.28.8.995
50. Mahon J, Blair GE, Wood GM, Scott BB, Losowsky MS, Howdle PD. Is persistent adenovirus 12 infection involved in celiac disease? A search for viral DNA using the polymerase chain reaction. Gut (1991) 32:1114–6. doi: 10.1136/gut.32.10.1114
51. Fine KD, Ogunji F, Saloum Y, Beharry S, Crippin J, Weinstein J. Celiac sprue: another autoimmune syndrome associated with hepatitis C. Am J Gastroenterol. (2001) 96:138–45. doi: 10.1111/j.1572-0241.2001. 03464.
52. Carlsson AK, Lindberg BA, Bredberg AC, Hyöty H, Ivarsson SA. Enterovirus infection during pregnancy is not a risk factor for celiac disease in the offspring. J Pediatr Gastroenterol Nutr. (2002) 35:649-52. doi: 10.1097/00005176-200211000-00011
53. Villalta D, Girolami D, Bidoli E, Bizzaro N, Tampoia M, Liguori M, et al. High prevalence of celiac disease in autoimmune hepatitis detected by antitissue tranglutaminase autoantibodies. J Clin Lab Anal. (2005) 19:6–10. doi: 10.1002/jcla.20047
54. Ruggeri C, La Masa AT, Rudi S, Squadrito G, Di Pasquale G, Maimone S, et al. Celiac disease and non-organ-specific autoantibodies in patients with chronic hepatitis C virus infection. Dig Dis Sci. (2008) 53:2151–5. doi: 10.1007/s10620-007-0146-1
55. Sarmiento L, Galvan JA, Cabrera-Rode E, Aira L, Correa C, Sariego S, et al. Type 1 diabetes associated and tissue transglutaminase autoantibodies in patients without type 1 diabetes and celiac disease with confirmed viral infections. J Med Virol. (2012) 84:1049–53. doi: 10.1002/jmv.23305
56. Tjernberg AR, Ludvigsson JF. Children with celiac disease are more likely to have attended hospital for prior respiratory syncytial virus infection. Dig Dis Sci. (2014) 59:1502–8. doi: 10.1007/s10620-014-3046-1
57. Abid SG, Aboud RS, Fadil HY, Aboud AS. Relationship between chronic hepatitis B virus and pathogenicity of celiac disease in the Iraqi patients. J Pharm Chem Biol Sci. (2016) 3:578–83.
58. Tarish HR, Hameed WS, Abdul-Mehdi RJ, Alsherees HAA. Role of previous adenovirus infection and its association with IFN-α in occurrence of celiac disease in Iraqi patients. J Med Sci Clin Res. (2016) 4:10326–30. doi: 10.18535/jmscr/v4i4.58
59. Alaedini A, Lebwohl B, Wormser GP, Green PH, Ludvigsson JF. Borrelia infection and risk of celiac disease. BMC Med. (2017) 15:169. doi: 10.1186/s12916-017-0926-1
60. Bouziat R, Hinterleitner R, Brown JJ, Stencel-Baerenwald JE, Ikizler M, Mayassi T, et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science (2017) 356:44–50. doi: 10.1126/science.aah5298
61. Myléus A, Hernell O, Gothefors L, Hammarström ML, Persson LÅ, Stenlund H, et al. Early infections are associated with increased risk for celiac disease: an incident case-referent study. BMC Pediatr. (2012) 12:194. doi: 10.1186/1471-2431-12-194
62. Kemppainen KM, Lynch KF, Liu E, Lönnrot M, Simell V, Briese T, et al. TEDDY Study Group. Factors that increase risk of celiac disease autoimmunity after a gastrointestinal infection in early life. Clin Gastroenterol Hepatol. (2017) 15:694–702. doi: 10.1016/j.cgh.2016.10.033
63. Mårild K, Kahrs CR, Tapia G, Stene LC, Størdal K. Infections and risk of celiac disease in childhood: a prospective nationwide cohort study. Am J Gastroenterol. (2015) 110:1475-84. doi: 10.1038/ajg.2015.287
64. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA. (2010) 107:11971–5. doi: 10.1073/pnas.1002601107

65. McLean MH, Dieguez D Jr., Miller LM, Young HA. Does the microbiota play a role in the pathogenesis of autoimmune diseases? Gut (2015) 64:332–41. doi: 10.1136/gutjnl-2014-308514
66. Thysen AH, Larsen JM, Rasmussen MA, Stokholm J, Bønnelykke K, Bisgaard H, et al. Prelabor cesarean section bypasses natural immune cell maturation. J Allergy Clin Immunol. (2015) 136:1123–5. doi: 10.1016/j.jaci.2015.04.044
67. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics (2006) 118:511–21. doi: 10.1542/peds.2005-2824
68. Kristensen K, Henriksen L. Cesarean section and disease associated with immune function. J Allergy Clin Immunol. (2016) 137:587–90. doi: 10.1016/j.jaci.2015.07.040
69. Mårild K, Stephansson O, Montgomery S, Murray JA, Ludvigsson JF. Pregnancy outcome and risk of celiac disease in offspring: a nationwide case-control study. Gastroenterology (2012) 142:39–45. doi: 10.1053/j.gastro.2011.09.047
70. Emilsson L, Magnus MC, Stordal K. Perinatal risk factors for development of celiac disease in children, based on the prospective Norwegian Mother and Child Cohort Study. Clin Gastroenterol Hepatol. (2015) 13:921–7. doi: 10.1016/j.cgh.2014.10.012
71. Sevelsted A, Stokholm J, Bønnelykke K, Bisgaard H. Cesarean section and chronic immune disorders. Pediatrics (2015) 135:e92–8. doi: 10.1542/peds.2014-0596
72. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Catassi C, SIGENP Working Group of Weaning and CD Risk. Mode of delivery and risk of celiac disease: risk of celiac disease and age at gluten introduction cohort study. J Pediatr. (2017) 184:81–6. doi: 10.1016/j.jpeds.2017.01.023
73. Koletzko S, Lee HS, Beyerlein A, Aronsson CA, Hummel M, Liu E, et al. TEDDY Study Group. Cesarean section on the risk of celiac disease in the offspring: the Teddy study. J Pediatr Gastroenterol Nutr. (2018) 66:417–24. doi: 10.1097/MPG.0000000000001682
74. Dydensborg Sander S, Hansen AV, Størdal K, Andersen AN, Husby S. Mode of delivery is not associated with celiac disease. Clin Epidemiol. (2018) 10:323–32. doi: 10.2147/CLEP.S152168
75. Mårild K, Ye W, Lebwohl B, Green PH, Blaser MJ, Card T, et al. Antibiotic exposure and the development of celiac disease: a nationwide case-control study. BMC Gastroenterol. (2013) 13:109. doi: 10.1186/1471-230X-13-109
76. Canova C, Zabeo V, Pitter G, Romor P, Baldovin T, Zanotti R, et al. Association of maternal education, early infections, and antibiotic use with celiac disease: a population-based birth cohort study in northeastern Italy. Am J Epidemiol. (2014) 180:76–85. doi: 10.1093/aje/kwu101
77. Galipeau HJ, McCarville JL, Huebener S, Litwin O, Meisel M, Jabri B, et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. Am J Pathol. (2015) 185:2969–82. doi: 10.1016/j.ajpath.2015.07.018
78. Olivares M, Neef A, Castillejo G, Palma GD, Varea V, Capilla A, et al. The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing celiac disease. Gut. (2015) 64:406–17. doi: 10.1136/gutjnl-2014-306931
79. Palma GD, Capilla A, Nova E, Castillejo G, Varea V, Pozo T, et al. Influence of milk-feeding type and genetic risk of developing celiac disease on intestinal microbiota of infants: the PROFICEL study. PLoS ONE (2012) 7:e30791. doi: 10.1371/journal.pone.0030791
80. Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalance in the composition of the duodenal microbiota of children with celiac disease. J Med Microbiol. (2007) 56:1669–74. doi: 10.1099/jmm.0.47410-0

81. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalances in faecal and duodenal Bifidobacterium species composition in active and non-active celiac disease. BMC Microbiol. (2008) 8:232. doi: 10.1186/1471-2180-8-232
82. Sanz Y, De Palma G, Laparra M. Unraveling the ties between celiac disease and intestinal microbiota. Int Rev Immunol. (2011) 30:207–18. doi: 10.3109/08830185.2011.599084
83. Olivares M, Castillejo G, Varea V, Sanz Y. Double- blind, randomised, placebo-controlled intervention trial to evaluate the effects of Bifidobacterium longum CECT 7347 in children with newly diagnosed celiac disease. Br J Nutr. (2014) 112:30–40. doi: 10.1017/S0007114514000609
84. Olivares M, Walker AW, Capilla A, Benítez-Páez A, Palau F, Parkhill J, et al. Gut microbiota trajectory in early life may predict development of celiac disease. Microbiome (2018) 6:36. doi: 10.1186/s40168-018-0 415-6
85. Leonard MM, Camhi S, Huedo-Medina TB, Fasano A. Celiac disease genomic, environmental, microbiome, and metabolomic (CDGEMM) study design: approach to the future of personalized prevention of celiac disease. Nutrients (2015) 7:9325–36. doi: 10.3390/nu7115470
86. Virta LJ, Kaukinen K, Collin P. Incidence and prevalence of diagnosed celiac disease in Finland: results of effective case finding in adults. Scand J Gastroenterol. (2009) 44:933–8. doi: 10.1080/00365520903030795
87. Catassi C, Kryszak D, Louis-Jacques O, Duerksen DR, Hill I, Crowe SE, et al. Detection of Celiac disease in primary care: a multicenter casefinding study in North America. Am J Gastroenterol. (2007) 102:1454–60. doi: 10.1111/j.1572-0241.2007.01173.x
88. Rosen A. Usefulness of symptoms to screen for celiac disease? Pediatrics (2014) 133. doi: 10.1542/peds.2012-3765
89. Hujoel IA, v Dyke CT, Brantner T, Larson J, King KS, Sharma A, et al. Natural history and clinical detection of undiagnosed celiac disease in a North American community. Aliment Pharmacol Ther. (2018) 47: 1358–66. doi: 10.1111/apt.14625
90. Kivelä L, Kaukinen K, Huhtala H, Lähdeaho ML, Mäki M, Kurppa K. At-risk screened children with celiac disease are comparable in disease severity and dietary adherence to those found because of clinical suspicion: a large cohort study. J Pediatr. (2017) 183:115–21. doi: 10.1016/j.jpeds.2016.12.077
91. Singh P, Arora S, Lal S, Strand TA, Makharia GK. Risk of celiac disease in the first- and second degree relatives of patients with celiac disease: a systematic review and meta-analysis. Am J Gastroenterol. (2015) 110:1539– 48. doi: 10.1038/ajg.2015.296
92. Elfström P, Sundström J, Ludvigsson JF. Systematic review with metaanalysis: associations between celiac disease and type 1 diabetes. Aliment Pharmacol Ther. (2014) 40:1123–32. doi: 10.1111/apt.12973
93. Sud S, Marcon M, Assor E, Palmert MR, Daneman D, Mahmud FH. Celiac disease and pediatric type 1 diabetes: diagnostic and treatment dilemmas. Int J Pediatr Endocrinol. (2010) 2010:161285. doi: 10.1155/2010/161285
94. Mahmud FH, De Melo EN, Noordin K, Assor E, Sahota K, Davies-Shaw J, et al. The celiac disease and diabetes-dietary intervention and evaluation trial (CD-DIET) protocol: a randomised controlled study to evaluate treatment of asymptomatic celiac disease in type 1 diabetes. BMJ Open (2015) 5:e008097. doi: 10.1136/bmjopen-2015-008097
95. vd Windt DA, Jellema P, Mulder CJ, Kneepkens CM, vd Horst HE. Diagnostic testing for CD among patients with abdominal symptoms: a systematic review. JAMA (2010) 303:1738–46. doi: 10.1001/jama.2010.549

96. Jansen M, Zelm M, Groeneweg M, Jaddoe V, Dik W, Schreurs M, et al. The identification of celiac disease in asymptomatic children: the Generation R Study. J Gastroenterol. (2018) 53:377–86. doi: 10.1007/s00535-017-1354-x
97. Choung RS, Murray JA. The US Preventive Services Task Force recommendation on screening for asymptomatic celiac disease: a dearth of evidence. JAMA (2017) 317:1221–3. doi: 10.1001/jama.2017.1105
98. Crowe SE. Celiac disease. Ann Intern Med. (2011) 154:2–16. doi: 10.7326/0003-4819-154-9-201105030-01005
99. Shamir R, Hernell O, Leshno M. Cost-effectiveness analysis of screening for celiac disease in the adult population. Med Decis Making (2006) 26:282–93. doi: 10.1177/0272989X06289012
100. Hershcovici T, Leshno M, Goldin E, Shamir R, Israeli E. Cost effectiveness of mass screening for celiac disease is determined by time-delay to diagnosis and quality of life on a gluten-free diet. Aliment Pharmacol Ther. (2010) 31:901–10. doi: 10.1111/j.1365-2036.2010.04242.x t
101. Nevoral J, Kotalova R, Hradsky O, Valtrova V, Zarubova K, Lastovicka J, et al. Symptom positivity is essential from omitting biopsy in children with suspected CD according to the new ESPGHAN guidelines. Eur J Pediatr. (2013) 173:497–502. doi: 10.1007/s00431-013-2215-0
102. Mansour AA, Najeeb AA. Celiac disease in Iraqi type 1 diabetic patients. Arab J Gastroenterol. (2011) 12:103–5. doi: 10.1016/j.ajg.2011.04.007
103. Björck S, Brundin C, Karlsson M, Agardh D. Reduced bone mineral density in children with screening-detected celiac disease. JPGN (2017) 65:526–32. doi: 10.1097/MPG.0000000000001568
104. Kurppa K, Paavola A, Collin P, Sievänen H, Laurila K, Huhtala H, et al. Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease. Gastroenterology (2014) 147:610–7.e7. doi: 10.1053/j.gastro.2014.05.003
105. van Koppen EJ, Schweizer JJ, Csizmadia CGDS, Krom Y, Hylkema HB, Van Geel AM, et al. Long-term health and quality-of life consequences of mass screening for childhood celiac disease: a 10-year follow-up study. Pediatrics (2009) 123:582–8. doi: 10.1542/peds.2008-2221
106. Webb C, Myleus A, Norstrom F, Hammarroth S, Hogberg L, Lagerqvist C, et al. High adherence to a gluten-free diet in adolescents with screening-detected celiac disease. JPGN (2015) 60:54–9. doi: 10.1097/MPG.0000000000000571
107. Vilppula A, Kaukinen K, Luostarinen L, Krekelä I, Patrikainen H, Valve R, et al. Clinical benefit of gluten-free diet in screen-detected older celiac disease patients. BMC Gastroenterol. (2011) 11:136. doi: 10.1186/1471-230X-11-136
108. Johnston SD, Rodgers C, Watson RG. Quality of life in screendetected and typical celiac disease and the effect of excluding dietary gluten. Eur J Gastroenterol Hepatol. (2004) 16:1281–6. doi: 10.1097/00042737-200412000-00008
109. Burger JPW, de Brouwer B, IntHout J, Wahab PJ, Tummers M, Drenth JPH. Systematic review with meta-analysis: dietary adherence influences normalization of health-related quality of life in celiac disease. Clin Nutr. (2017) 36:399–406. doi: 10.1016/j.clnu.2016.04.021
110. Dennis M, Case S. Going gluten-free: a primer for clinicians. Pract Gastroenterol. (2004) 28:86–104.
111. Lee AR, Ng DL, Dave E, Ciaccio EJ, Green PH. The effect of substituting alternative grains in the diet on the nutritional profile of the gluten-free diet. J Hum Nutr Diet. (2009) 22:359–63. doi: 10.1111/j.1365-277X.2009.00970.x
112. Wessels MMS, Te Lintelo M, Vriezinga SL, Putter H, Hopman EG, Mearin ML. Assessment of dietary compliance in celiac children using a standardized dietary interview. Clin Nutr. (2018) 37:1000–4. doi: 10.1016/j.clnu.2017.04.010

113. Vriezinga S, Borghorst A, van den Akker-van Marle E, Benninga M, George E, Hendriks D, et al. E-healthcare for celiac disease–a multicenter randomized controlled trial. J Pediatr. (2018) 195:154–60. doi: 10.1016/j.jpeds.2017.10.027
114. Hogen Esch CE, Wolters VM, Gerritsen SA, Putter H, von Blomberg BM, van Hoogstraten IM, et al. Specific celiac disease antibodies in children on a gluten free diet. Pediatrics (2011) 128:547–52. doi: 10.1542/peds.2010-3762
115. Roos S, Wilhelmsson S, Hallert C. Swedish women with celiac disease in remission use more health care services than other women: a controlled study. Scand J Gastroenterol. (2011) 46:13–9. doi: 10.3109/00365521.2010.516448
116. Rubio-Tapia A, Rahim M, See J, Lahr B, Wu T, Murray J. Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. Am J Gastroenterol. (2010) 105:1412–20. doi: 10.1038/ajg.2010.10
117. Moreno ML, Cebolla Á, Muñoz-Suano A, Carrillo-Carrion C, Comino I, Pizarro Á, et al. Detection of gluten immunogenic peptides in the urine of patients with celiac disease reveals transgressions in the gluten-free diet and incomplete mucosal healing. Gut (2017) 66:250–7. doi: 10.1136/gutjnl-2015-310148
118. Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in celiac disease subjects: a proof of concept study. Aliment Pharmacol Ther. (2007) 26:757–66. doi: 10.1111/j.1365-2036.2007.03413.x
119. Gopalakrishnan S, Durai M, Kitchens K, Tamiz AP, Somerville R, Ginski M, et al. Larazotide acetate regulates epithelial tight junctions in vitro and in vivo. Peptides (2012) 35:86–94. doi: 10.1016/j.peptides.2012.02.015
120. Leffler DA, Kelly CP, Abdallah HZ, Colatrella AM, Harris LA, Leon F, et al. A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge. Am J Gastroenterol. (2012) 107:1554–62. doi: 10.1038/ajg.2012.211
121. Leffler DA, Kelly CP, Green PH, Fedorak RN, DiMarino A, Perrow W, et al. Larazotide acetate for persistent symptoms of celiac disease despite a glutenfree diet: a randomized controlled trial. Gastroenterology. (2015) 148:1311–9. doi: 10.1053/j.gastro.2015.02.008
122. Kelly CP, Green PH, Murray JA, Dimarino A, Colatrella A, Leffler DA, et al. Larazotide Acetate Celiac Disease Study Group. Larazotide acetate in patients with celiac disease undergoing a gluten challenge: a randomised placebo-controlled study. Aliment Pharmacol Ther. (2013) 37:252–62. doi: 10.1111/apt.12147
123. Gass J, Bethune MT, Siegel M, Spencer A, Khosla C. Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue. Gastroenterology (2007) 133:472–80. doi: 10.1053/j.gastro.2007.05.028
124. Bethune MT, Strop P, Tang Y, Sollid LM, Khosla C. Heterologous expression, purification, refolding, and structural-functional characterization of EP-B2, a self-activating barley cysteine endoprotease. Chem Biol. (2006) 13:637–47. doi: 10.1016/j.chembiol.2006.04.008
125. Murray JA, Kelly CP, Green PHR, Marcantonio A, Wu TT, Mäki M, et al. CeliAction Study Group of Investigators. No difference between Latiglutenase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. Gastroenterology (2017) 152:787–98. doi: 10.1053/j.gastro.2016.11.004
126. Syage JA, Murray JA, Green PHR, Khosla C. Latiglutenase improves symptoms in seropositive celiac disease patients while on a glutenfree diet. Dig Dis Sci. (2017) 62:2428–32. doi: 10.1007/s10620-017-4 687-7

127. Stepniak D, Spaenij-Dekking L, Mitea C, Moester M, de Ru A, Baak-Pablo R, et al. Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. Am J Physiol Gastrointest Liver Physiol. (2006) 291:G621–G629. doi: 10.1152/ajpgi.00034.2006
128. König J, Holster S, Bruins MJ, Brummer RJ. Randomized clinical trial: effective gluten degradation by Aspergillus niger-derived enzyme in a complex meal setting. Sci Rep. (2017)7:13100. doi: 10.1038/s41598-017-13587-7
129. Goel G, King T, Daveson AJ, Andrews JM, Krishnarajah J, Krause R, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in celiac disease: two randomised, double-blind, placebo-controlled phase 1 studies. Lancet Gastroenterol Hepatol. (2017) 2:479–93. doi: 10.1016/S2468-1253(17)30110-3

4

Early diagnosis of celiac disease in the Preventive Youth Health Care Centres in the Netherlands: study protocol of a case-finding study (GLUTENSCREEN)

BMJ Pediatrics Open 2021 Aug 11;5(1):e001152

Caroline R. Meijer
M. Elske van den Akker
Leti van Bodegom
Johanna C. Escher
Nan van Geloven
Floris van Overveld
Edmond H.H.M. Rings
Lucy Smit
Martine de Vries
M. Luisa Mearin

ABSTRACT

Introduction: Celiac disease (CD) occurs in 1% of the population, develops early in life and is severely underdiagnosed. Undiagnosed and untreated disease is associated with short- and long-term complications. The current health care approach is unable to solve the underdiagnosis of CD and timely diagnosis and treatment is only achieved by active case-finding. Aim: to perform a case-finding project to detect CD children who visit the Youth Health Care Centres (YHCCs) in a well-described region in the Netherlands to show that it is feasible, cost-effective and well accepted by the population.

Methods/analysis: Prospective intervention cohort study. Parents of all children aged 12 months-4 years attending the YHCCs for a regular visit are asked if their child has one or more CD-related symptoms from a standardized list. If so, they will be invited to participate in the case-finding study. After informed consent, a point of care test (POCT) to assess CD-specific antibodies against tissue-transglutaminase (TG2A), is performed onsite the YHCCs. If the POCT is positive, CD is highly suspected and the child will be referred to hospital for definitive diagnosis according to the ESPGHAN guideline.
Main outcomes: 1. incidence rate of new CD diagnoses in the study-region in comparison to the rest of the Netherlands.
2. Feasibility and cost-effectiveness of active CD-case-finding at the YHCCs. All costs of active case- finding, diagnostics and treatment of CD and the potential short- and long-term consequences of the disease will be calculated for the setting with and without case-finding.
3. Ethical acceptability: by questionnaires on parental and healthcare professionals' satisfaction.
A statistical analysis plan (SAP) has been written and will be published on the GLUTEN-SCREEN-website.

Ethics and dissemination: The Medical Ethics Committee Leiden approved this study. If we prove that case-finding at the YHCC is feasible, cost-effective and well accepted by the population, implementation is recommended.

INTRODUCTION

Celiac Disease (CD) is an immune-mediated systemic disorder elicited by the ingestion of gluten containing cereals from the normal diet (among others wheat, rye and barley) in genetically susceptible individuals. CD is characterized by a variable combination of gluten-dependent clinical manifestations, CD specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes and enteropathy[1, 2]. CD has a frequency of at least 1% in the general population, i.e., 168,000 individuals and 33,600 children in the Netherlands[3-6]. It is the most common food intolerance in the Netherlands and therefore a significant public health problem. CD is frequently unrecognized, partially because of its variable clinical presentations and symptoms, ranging from malabsorption with chronic diarrhea, poor growth in children and weight loss, to nonspecific signs and symptoms like chronic fatigue, osteoporosis/reduced bone mineral density, iron-deficiency anaemia, anorexia, chronic abdominal pain, vomiting, flatulence, irritability, elevated liver enzymes or constipation[1, 7]. CD has a considerable health burden for society. In addition to the signs and symptoms, untreated disease is associated with long-term complications such as delayed puberty, neuropsychiatric disturbances, associated autoimmune disease, miscarriages, small-for-date-births, osteoporosis, and, rarely, malignancy[1, 8]. CD increases the overall mortality risk, reduces the quality of life (QoL) and yields extensive negative economic consequences, thereby presenting a resource challenge for current and future health systems[9, 10, 11].

In 1999 our research group published that childhood CD in the Netherlands was severely underdiagnosed: for every child diagnosed with CD, there were seven who have unrecognized, and therefore untreated disease[12]. Data from the National Dutch Pediatric Surveillance Unit (DPSU) show 1107 new cases in 2010-2013 of clinically diagnosed CD in children 0-14 years[13, 14]. The percentage of children diagnosed with CD <2 years of age was 30%, and < 4 years of age was 50%. Those were also the children with the most severe clinical presentations[13, 14].

DPSU is a unique registry of the Dutch Society of Pediatrics, comprising of all Dutch pediatric practices. Under it, Dutch pediatricians are asked to report newly diagnosed cases of certain diseases (CD in our case). DPSU respondents have a 90% mean response rate. The incidence of 1.56/1000 live births in 2010-2013 does not correspond to the prevalence in the general population [13, 15]. This illustrates that the current standard health care is not able to solve the problem. Once diagnosed, the patient's health status improves after treatment with a gluten free diet (GFD), but prevention would be more beneficial[7, 16].

Results from recent prospective studies have shown that primary prevention of CD by improving the timing of gluten introduction and/or the duration or maintenance of breast-feeding is not possible[17-21]. For this reason, early diagnosis and treatment of CD represents the only way to (secondary) prevention. There are two approaches to achieve this: mass screening and case-finding. The Medical Ethics Committee (METC-Leiden Den Haag Delft, METC-LDD) considered the current evidence insufficient to assess the balance of benefits and harms of screening for CD in asymptomatic children (mass screening),[22, 23]. Consequently, we propose an active case finding project in symptomatic children in a Youth Health Care Centres (YHCC) region in the Netherlands to achieve secondary prevention of the disease. Active case-finding refers to liberal diagnostic testing of patients with CD-associated symptoms. In the general adult population, this approach has led to the early diagnosis of a large number of patients, resulting in significantly health improvement after treatment, good compliance with the GFD and good CD related QoL[24, 25].

In the Netherlands, more than 95% of all children 0 months-4 years visit the YHCCs[26]. The goal of YHC is to promote and secure the health and safety of all children 0-18 years,[27]. YHC aims at primary and secondary prevention of diseases in order to promote healthy growth and development. Secondary prevention (early diagnosis and treatment) of CD therefore fits within the goals of YHC. The validated, rapid point of care test (POCT) to determine CD specific antibodies represent a reliable, cheap, and easy-to-use instrument for CD case-finding in children[28]. Therefore, early detection of CD by case finding in the YHCCs offers a “window of opportunity” to identify CD as soon as possible preventing more severe symptoms and complications of the disease.

Aims and hypothesis

The aim of the present study is to perform a novel case-finding project to detect CD in 12 months-to 4 years old children who visit the YHCCs in a well-described region in the Netherlands, to show that it is feasible, cost-effective and well accepted by the population. We hypothesize that GLUTENSCREEN is feasible, cost-effective and well-acceptable by the general population. To achieve this, GLUTENSCREEN will compare the results of the case-finding strategy to the outcome of current healthcare in the diagnosis of CD in children in the rest of the country.

METHODS AND ANALYSIS

Study design

The study is a prospective intervention cohort study. The project started the 4th of February 2019 and will end the 1st of February 2023 (with interruption of 5 months due to the COVID pandemic). All parents of children aged 12 months-4 years attending scheduled visits to the YHCCs in the region Midden and Zuid Kennemerland, to be further called "Kennemerland" will be informed. At the YHCC a standardized questionnaire on CD-related symptoms will be checked (annex 1). Symptoms are reported by the parents. Weight and growth are controlled at the YHCC. If one or more CD-associated symptoms (including growth restrictions) are present, the child is eligible for the study. The CD-related symptoms (see annex 1) are based on the recommendations of CD testing (taking into account the absence of previous laboratory or other investigations, and the age of the project population) in symptomatic children and adolescents in the Guideline Celiac Disease of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[1].

Control population

A national control group is based on the data reported by DPSU. Dutch pediatricians are asked by the DPSU to report newly diagnosed cases of certain diseases (CD in our case) monthly during the time of this case-finding project. The CD cases are clinically diagnosed by the pediatricians to the current standard of care. DPSU respondents have a 90% mean response rate. The cases of clinically diagnosed CD in the study region will be identified by the data of the YHCC.

Inclusion and exclusion criteria

Inclusion criteria are: 1. 12 months to 4 years of age, 2. following a gluten containing diet, 3. one or more CD-associated symptoms (annex 1), 4. parents have a sufficient knowledge of Dutch language, 5. informed consent. Exclusion criterium: 1. diagnosed with CD

Recruitment and procedure

Eligible children will be identified by the YHCC administration. During 2.5 years, the parents/legal guardians (from this point on called "parents") will receive an advance invitation from the YHCC Kennemerland with information about the study. During the regularly scheduled visit at the YHCC, the nurse or the doctor will check the symptoms list (annex 1); if one or more CD-associated symptoms are present, the nurse/doctor will give the parents the information letter and informed consent form and, after informed consent is given, she/he will make a new appointment to perform the POCT. The POCT

for TG2A will be performed. The symptoms list and informed consent form will be stored in a separate file in the child's electronic record.

Intervention

After informed consent a validated POCT to determine CD specific antibodies (TG2A, Celiac Quick Test; BioHit Oyj, Finland) which is also suitable for Immunoglobulin A (IgA)- deficient patients will be performed. It requires 1 drop of fresh blood, obtained by finger-prick. The result (positive/negative) should be interpreted after 10 minutes. If the result is negative (no TG2A) the child is considered to not have CD and the procedure is finished for this child. If the POCT is positive, the child will be referred to the pediatric-gastroenterologist for further investigation for CD diagnosis at the Outpatient Clinic of the Department of Pediatric-Gastroenterology of the Leiden University Medical Center (LUMC) in the following 3 weeks. In the LUMC, CD will be diagnosed according to the ESPGHAN guidelines,[1, 2]. A second visit (face-to-face or by telephone, depending on parental preference) will be scheduled 14 days later to discuss results. There are 3 possible outcomes:

1. CD ruled out: No further follow-up is needed.
2. CD likely, but unproven; diagnostic duodenal biopsies are advised.
3. CD is diagnosed. The patient/parents will be counselled on treatment and follow-up.

If an endoscopy to obtain duodenal biopsies under general anaesthesia is advised, the parents will receive written information on the procedure, as all other parents do in the outpatient clinic when this procedure is advised. Parents have to give oral informed consent for this procedure, and this will be noted in the patient's medical record. The procedure will be carried out per usual LUMC regulations. Biopsies will only be performed when medically indicated for the child and not just for purpose of scientific research.

Training and protocol adherence

To perform the POCT, the YHCC healthcare professionals followed a training provided by the employees of BioHit and according to the manufacturer's instructions. To prevent protocol drifting they receive monthly supervision by a senior clinical physician. All POCT results are photographed and stored in the electronic patient's file. Monthly, the researchers and the senior clinical physician of the YHCC evaluate the organization, procedure and results.

Outcome measures

The main study outcomes are:

1. The incidence rate of new CD diagnoses in the study region Kennemerland in comparison to the rest of the Netherlands.

2. Cost-effectiveness of active case-finding of CD in the YHCCs compared to standard care.
3. Ethical acceptability: by questionnaires on parental satisfaction and health care professionals.

Data collection

The result of the POCT will be noticed in the medical file as well as the diagnosis after further investigation. Diagnostic tools and consultations after a positive POCT will be noticed in a database and in the medical file of the child.

Parents of children who visit the YHCC and/or participate in GLUTENSCREEN, will be asked to fill in standardized questionnaires on their opinion regarding the actual case-finding and on mass screening for CD. We will ask the opinion of 1) Parents of asymptomatic children, (by definition excluded for participation in case-finding); 2) Parents who decline participation in the study; 3) Parents participating in the case-finding and 4) Parents of children with suspected CD by the case-finding procedure who will be referred to the hospital for definitive CD diagnosis. Also, the health care professionals in the YHCCs with various tasks within GLUTENSCREEN will also be asked to give their opinion about the case-finding.

Costs of active case-finding, diagnostics and treatment of CD and they will be compared to the costs of diagnostics and treatment of standard care. The costs of active case-finding are the costs of discussing the symptoms list, measurement of TG2A by POCT and the diagnostic costs after a positive test (repeated TG2A measurement, endomysium antibodies (EMA), human leucocyte antigen (HLA)-typing, biopsy, pediatric consultation etc.). These costs will be measured in the prospective intervention cohort study. Cost of measurement of TG2A levels include time needed from YHC professionals and cost of test equipment and materials. Resource use after a positive test will be measured by means of a case record form. Information on diagnostic procedures of clinically diagnosed CD will be collected by the DPSU and the Dutch Celiac Society (NCV), supplemented with parent questionnaires on healthcare use outside the hospital. Health care use will be valued according to the Dutch guideline for costing research[29].

In addition, an estimate for the costs of long-term consequences of undiagnosed CD as delayed puberty, neuropsychiatric disturbances, dental enamel hypoplasia, associated autoimmune diseases, miscarriages, small for date-births, osteoporosis, and (rarely) malignancy will be made based on literature. Together with the comparison of the cost of diagnosis and treatment of CD between a situation with and without case finding,

this will give an estimate for the cost-effectiveness of active case-finding compared to standard care for a lifetime horizon.

Withdrawal

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. The parents of children who withdraw are asked to fill in the questionnaire on acceptability.

Sample size

We assume that in the Dutch population outside the case-finding project, the incidence of children 1-4 years old with a diagnosis of CD equals 0.62/1000 children's years. With 2.5 years inclusion period, we expected 5434 children taking the POCT would give high power (about 95%) to detect an at least two times higher incidence rate in the study region (alpha 5%). We expected 60% of the children to be symptomatic, and 60% participation of those symptomatic children in the POCT, so 15,100 children would need to be requested for participation, in order to obtain 5434 children available for case-finding using a rapid POCT. Since the population in the YHCCs in the Kennemerland region is approximately 12,000 children/year with additional 4,000 added per year and 2.5 years of study duration was considered sufficient to achieve sufficient sample size. When in March 2020 the study had to be interrupted for 5 months due to the COVID pandemic, the sample size calculation was re-evaluated based on the results up to that moment, including the number of cases found in the study region in the first year of the study. Based on this evaluation, it was decided that the original inclusion period of 2.5 years could be retained.

Statistical analyses

For the primary analysis, the incidence rate in the case-finding population will be calculated along with a 95% confidence interval and will be compared with the incidence rate in the Netherlands, obtained from the DPSU, in the same period assuming the latter has no sampling variability (so using the incidence rate in the rest of the Netherlands as a fixed reference value).

All costs of active case finding, diagnostics and treatment of CD and the potential short-term consequences of the disease will be calculated for the setting with and for the cost-effectiveness without active case finding. Healthcare use will be valued according to the Dutch guideline for costing research. For the acceptability descriptive and univariate logistic regression analyses will be performed comparing the answers from the

different groups. Also, univariable logistic regression analysis of negative feelings and POCT-result in relation to acceptability will also be done.

Ethics approval

The study is approved by the Medical Ethics Committee of the Leiden University Medical Centre. All study data will be handled confidentially and coded with a unique study number. Only the research team will have access to the data. A data management plan is available.

DISCUSSION

Several studies have shown that an active case-finding strategy in the primary care setting is an effective means to improve the (early) diagnostic rate of CD and to achieve secondary prevention[24, 25].

National guidelines on the diagnosis and treatment of CD published in 2008 recommend testing for CD in patients with a wide spectrum of intestinal and extra intestinal manifestations, in asymptomatic family members of CD cases and in groups with related conditions. This approach, together with the availability of reliable CD antibody tests, have led to a rise in the incidence of diagnosed CD in Dutch children from 1.21/1000 live births in 2000 to 1.56/1000 live births in 2010-2013. Nevertheless, the increased incidence rate does not closely correspond to its frequency in the general population. In the Generation-R project, a population based prospective cohort study, the prevalence of CD at 6 years of age was 1.5%. Due to the shift in CD presenting symptoms towards a milder form, the delay from first symptoms to CD diagnosis has been reported to be unacceptably long, at between 5–10 years for many persons and so the need for earlier diagnosis has been advocated. Early diagnosis is expected to reduce serious clinical CD. Data from the DPSU shows that 50% of the 1107 new cases of clinically diagnosed CD in children aged 0-14 years between January 2010 and December 2013 were < 4 years. These young children had the most severe symptoms of CD, including chronic diarrhoea and weight loss (71.0%) or wasting/failure to thrive (65.9%),[13, 14]. Therefore, with active case finding we aim to prevent the most serious manifestations of childhood CD.

Our study has several strengths: first, to the best of our knowledge, this is the first initiative for active case finding in the general population in the Netherlands. Since the majority of the children aged 1-4 years visit the YHCC, the study will provide insight into the incidence of childhood CD in symptomatic children in the Netherlands. Second, the actual health costs of the diagnosis of childhood CD and the cost-effectiveness of active case-finding in the Netherlands have never been prospectively investigated. Third, this study will provide important information about the acceptability of the general Dutch

population concerning active case finding and in addition about the willingness of parents of asymptomatic children to participate in a mass screenings project on CD.

It would also have been interesting to explore the possibility of HLA determination at the YHCCs. Since more than 95% of CD patients carry these HLA haplotypes, their presence is valuable in identifying the population that may develop CD. In the Netherlands, about 40% of the general population is HLA DQ2 or DQ8 positive and the presence of these haplotypes is thus not discriminative for the disease. On the other hand, repeated CD testing will be unnecessary in HLA-DQ2/DQ8 negative individuals. However, HLA-DQ typing currently present important drawbacks for it to be used outside the hospital. There are no rapid tests since DNA preparation takes time. Material for DNA extraction can be obtained from whole blood (minimum quantity 4-5 ml) or from other cells, such as cheek mucosa. Venepunctures are not feasible at YHCCs. Obtaining cheek cells by smoothly brushing the buccal mucosa is a possibility, but the necessary mechanisms to store and transport the material poses logistical and economic challenges. The costs of transport, DNA extraction, HLA-typing and distribution of tests results are likely to increase the costs of the active case-finding.

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr 2012;54:136–60.
2. Husby S, Koletzko S, Korponay-Szabó I, et al. European Society pediatric gastroenterology, hepatology and nutrition guidelines for diagnosing celiac disease 2020. J Pediatr Gastroenterol Nutr 2020;70:141–56.
3. Singh P, Arora A, Strand TA, et al. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2018;16:823–36.
4. Steens RFR, Csizmadia CGDS, George EK, 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 2005;147:239–43.
5. George EK, Mearin ML, van der Velde EA, et al. Low incidence of childhood celiac disease in the Netherlands. Pediatr Res 1995;37:213–8.
6. Jansen MAE, Kiefte-de Jong JC, Gaillard R, et al. Growth trajectories and bone mineral density in anti-tissue transglutaminase antibody-positive children: the generation R study. Clin Gastroenterol Hepatol 2015;13:913–20.
7. Hogen Esch CE, Kiefte-de Jong J, Hopman E. Strategies for prevention of celiac disease. frontiers in celiac disease. Pediatr Adolesc Med 2008;12:188–97.
8. Kiefte-de Jong JC, Jaddoe VWV, Uitterlinden AG, et al. Levels of antibodies against tissue transglutaminase during pregnancy are associated with reduced fetal weight and birth weight. Gastroenterology 2013;144:726–35.
9. Biagi F, Corazza GR. Mortality in celiac disease. Nat Rev Gastroenterol Hepatol 2010;7:158–62.
10. van Doorn RK, Winkler LMF, Zwinderman KH, et al. CDDUX: a disease-specific health-related quality-of-life questionnaire for children with celiac disease. J Pediatr Gastroenterol Nutr 2008;47:147–52.
11. Shamir R, Hernell O, Leshno M. Cost-Effectiveness analysis of screening for celiac disease in the adult population. Med Decis Making 2006;26:282–93.
12. Csizmadia CG, Mearin ML, von Blomberg BM, et al. An iceberg of childhood celiac disease in the Netherlands. Lancet 1999;353:813–4.
13. Schweizer JJet al. The 3rd national survey on childhood celiac disease in the Netherlands: incidence and clinical presentation. JPGN 2013;56:PO-G-0030.
14. Meijer CR, Schweizer JJ, Peeters A, et al. Efficient implementation of the 'non-biopsy approach' for the diagnosis of childhood celiac disease in the Netherlands: a national prospective evaluation 2010-2013. Eur J Pediatr 2021.
15. Jansen M, van Zelm M, Groeneweg M, et al. The identification of celiac disease in asymptomatic children: the generation R study. J Gastroenterol 2018;53:377–86.
16. Meijer C, Shamir R, Szajewska H, et al. Celiac disease prevention. Front Pediatr 2018;6:368.
17. Vriezinga SL, Auricchio R, Bravi E, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med 2014;371:1304–15.
18. Lionetti E, Castellaneta S, Francavilla R, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med 2014;371:1295–303.
19. Størdal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132:e1202–9.

20. Aronsson CA, Lee H-S, Liu E, et al. Age at gluten introduction and risk of celiac disease. Pediatrics 2015;135:239–45.
21. Szajewska H, Shamir R, Chmielewska A, et al. Systematic review with meta-analysis:early infant feeding and celiac disease—update 2015. Aliment Pharmacol Ther 2015;41:1038–54.
22. Rosén A, Sandström O, Carlsson A, et al. Usefulness of symptoms to screen for celiac disease. Pediatrics 2014;133:211–8.
23. Chou R, Bougatsos C, Blazina I. Screening for celiac disease evidence report and systematic review for the US preventive services Task force. JAMA 2017;317:1252–7.
24. Virta LJ, Kaukinen K, Collin P. Incidence and prevalence of diagnosed celiac disease in Finland: results of effective case finding in adults. Scand J Gastroenterol 2009;44:933–8.
25. Catassi C, Kryszak D, Louis-Jacques O, et al. Detection of celiac disease in primary care: a multicenter case-finding study in North America. Am J Gastroenterol 2007;102:1454–60.
26. Inspectie voor de Gezondheidszorg. De jeugdgezondheidszorg beter in positie. Utrecht, 2014.
27. Nederlands Centrum Jeugdgezondheid. Landelijk professioneel kader, uitvoering basispakket jeugdgezondheidszorg, 2015. Available: www. ncj. nl
28. Korponay-Szabó IR, Szabados K, Pusztai J, et al. Population screening for celiac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study. BMJ 2007;335:1244–7.
29. Hakkaart-van Roijen L, Linden N, Bouwmans C. Handleiding voor kostenonderzoek, methoden en standaard kostprijzen voor economische evaluaties in de gezondheidszorg. Geactualiseerde versie, 2010.
30. Richtlijn Coeliakie en Dermatitis Herpetiformis. Available: https://www. mdl. nl/ files/ richlijnen/ richtlijn_ Coeliakie_ definitief. pdf

5

Prediction models for celiac disease development in children from high-risk families: Data from the PreventCD cohort

Gastroenterology 2022 Aug;163(2):426-436

Caroline R. Meijer*
Renata Auricchio*
Hein Putter
Gemma Castillejo
Paula Crespo
Judit Gyimesi
Corina Hartman
Sanja Kolacek
Sibylle Koletzko
Ilma R. Korponay-Szabo
Eva Martinez Ojinaga
Isabel Polanco
Carmen Ribes-Koninckx
Raanan Shamir
Hania Szajewska
Riccardo Troncone
Vincenzo Villanacci
Katharina Werkstetter
M. Luisa Mearin
*shared first authorship

Supplementary Appendix is available on 1-s2.0-S0016508522004425-mmc1.pdf (els-cdn.com)

ABSTRACT

BACKGROUND AND AIMS: Screening for celiac disease (CD) is recommended in children with affected first-degree relatives (FDR). However, the frequency of screening and at what age remain unknown. Aims: to detect variables influencing the risk of CD-development and develop and validate clinical prediction models to provide individualized screening advice.

METHODS: Analysis of prospective data from the ten years follow-up of the PreventCD-birth cohort involving 944 genetically predisposed children with CD-FDR. Variables significantly influencing the CD-risk were combined to determine a risk score. Landmark analyses were performed at different ages. Prediction models were created by multivariable Cox proportional hazards regression analyses, backward elimination and Harrell's c-index for discrimination. Validation was done using data from the independent NeoCel cohort.

RESULTS: In March 2019, the median follow-up was 8.3 years (22 days-12.0 years); 135/944 children developed CD (mean age 4.3 years (1.1-11.4). CD developed significantly more often in girls (p=0.005) and in HLA-DQ2 homozygous individuals (8-year cumulative incidence 35.4% versus maximum of the other HLA-risk groups 18.2% [p<0.001]). The effect of homozygosity DR3-DQ2/DR7-DQ2 on CD-developing was only present in girls (interaction p=0.04). The prediction models showed good fitting in the validation cohort (Cox regression 0.81(0.54)). To calculate a personalized risk of CD-development and provide screening advice, we designed the Prediction application https://hputter.shinyapps.io/preventcd/.

CONCLUSION: Children with CD-FDR develop CD early in life, and their risk depends on gender, age and HLA-DQ: all factors which are important for a sound screening advice. These children should be screened early in life, including HLA-DQ2/8-typing, and if genetically predisposed to CD, should get further personalized screening advice using our Prediction app.

INTRODUCTION

Celiac disease (CD) is a common autoimmune disorder caused by the ingestion of gluten in genetically susceptible individuals. It is characterized by CD-specific antibodies and HLA-DQ2 and/or HLA-DQ8 haplotypes.[1] CD affects as many as 1-3 % of the general population.[2,3] Among first-degree relatives (FDR) of CD patients, the disease prevalence is much higher, being approximately 10-20% depending on the HLA-DQ and gender.[4-6] This has been prospectively evaluated among others in the PreventCD cohort, consisting of 944 children with at least one FDR with CD and HLA-DQ2 and/or HLA-DQ8. The children were enrolled at birth between 2007 and 2010 in Croatia, Germany, Hungary, Israel, Italy, the Netherlands, Poland and Spain. Initially, a randomized, double-blind, placebo-controlled dietary intervention was performed and the results, published in 2014 in the New England Journal of Medicine, showed that the early introduction of small quantities of gluten and/or breastfeeding did not reduce the risk of CD at three years of age.[5] The data of the follow-up of the PreventCD cohort at the mean age of ten years offers a unique opportunity to study the natural development of CD in children from high-risk families. The aims of this study were, (i) to detect variables that influence the age-dependent risk of CD development in children with affected FDR, and (ii) to build clinically applicable prediction models for CD development among these children to allow for personalized advice for their CD screening.

MATERIALS AND METHODS

PREVENTCD-COHORT

CD diagnosis

Data was frozen on 29th March 2019. All children were assessed regularly from birth onwards for CD development at pre-defined intervals, including seven times during the first three years of age and thereafter annually or at least once between March 2016 and March 2019.[5] We monitored parent-reported health status, weight and height, gluten consumption (up until the age of three years, quantified using standardized questionnaires) and serum IgA against anti-transglutaminase (TGA) (Supplementary Appendix).

(Parents of) children with elevated TGA and/or CD symptoms suggestive of CD, were offered small bowel biopsies to confirm the diagnosis. The date of CD diagnosis was defined as the date of small bowel biopsy or as the date on which TGA were highest. Given that TGA were determined at variable intervals starting from 3 years of age, we considered the age of CD development to be midway between the age at which the last negative TGA was determined and the date of CD diagnosis.

The study was approved by all medical ethics committees of the participating centers. All the authors had access to the study data and had reviewed and approved the final manuscript.

Statistical methods

The statistical analysis plan (SAP) was published online on 29 March 2019 before the analyses were performed using R version 3.6.1 (Supplement 2, pages 83-90 and https://www.preventcd.com/images/stories/Downloads/2019-0402%20Statistical%20Analysis%20Plan_PreventCD_final.pdf). In case a child was lost to follow-up, the child was treated as censored on the date of last visit/TGA determination. For univariate comparison of cumulative incidences of CD between groups, the log-rank test (two-sided) was used.

Prediction models

To develop the models, all the factors that significantly influenced the risk of CD development were combined into a risk score.

Baseline model

Multivariable Cox proportional hazards regression analysis of the baseline was performed in two steps. In the first step, three primary variables already known at the child's birth (gender, HLA-risk group, number of affected FDR, table 1) were entered into the model, irrespective of statistical significance. In accordance with our previous publication, we analyzed the risk for CD in five groups according to HLA-DQ genotype (see Supplementary Appendix).[5] In addition, we also exploratively analyzed the risk for CD in children with DR3-DQ2/DR3-DQ2 separately from those with DR3-DQ2/DR7-DQ2, as the affinity of gluten peptides is higher for DR3-DQ2 than for DR7-DQ2 receptors.[7,8] Because of the low number of children with 3 or more affected FDRs (7), these were considered together in one category. The second step consisted of adding the secondary variables (country of origin, type of affected FDR, maternal diet, delivery mode and early intervention with gluten or placebo, table 1) to the model using backward elimination based on Akaike Information Criterion (AIC), thus guarding against overfitting.[9,10]

Landmark prediction models

Analyses for variables occurring after birth (duration of breastfeeding, duration of exclusive breastfeeding, rotavirus vaccination, infections as reported by parents and gluten intake) were performed at one, two and three years of age (infections until six years of age) (Supplementary Appendix). For each analysis, the information available at the landmark time point was used. Models' backward elimination based on AIC was used. Since quantification of daily gluten intake is usually unknown in the standard medi-

Table 1. Distribution of the baseline variables in the PreventCD cohort (n =944)

VARIABLE	VALUES	N (%)	TOTAL (%)	CD (%)	P-VALUE, UNIVARIATE ANALYSIS
PRIMARY VARIABLES					
1.Gender					0.005
	Male	490 (51.9)	944 (100)	56 (11.4)	
	Female	454 (48.1)		79 (17.4)	
2. HLA risk group*					<0.001
	Group 1	129 (14.2)	911 (96.5)	40 (31.0)	
	Group 2	88 (9.7)		14 (15.9)	
	Group 3	417 (45.8)		58 (13.9)	
	Group 4	66 (7.2)		8 (12.1)	
	Group 5	211 (23.2)		13 (6.2)	
3.Number of affected FDR					0.01
	1	863 (91.4)	944 (100)	115 (13.3)	
	2	74 (7.8)		19 (25.7)	
	3 or more	7 (0.7)		1 (14.3)	
SECONDARY VARIABLES					
4. Country					0.06
	Netherland	133 (14.1)	944 (100)	22 (16.5)	
	Italy	139 (14.7)		20 (14.4)	
	Poland	64 (6.8)		5 (7.8)	
	Spain	249 (26.4)		25 (10.0)	
	Germany	113 (12.0)		13 (11.5)	
	Israel	95 (10.1)		19 (20.0)	
	Croatia	13 (1.4)		0 (0)	
	Hungary	138 (14.6)		31 (22.5)	
5. Type of affected FDR					0.01
	Mother only	407 (43.1)	944 (100)	62 (15.2)	
	Father only	89 (9.4)		10 (11.2)	
	One sib only	367 (38.9)		43 (11.7)	
	Mother+sib(s)	46 (4.9)		15 (32.6)	
	Father+sib(s)	14 (1.5)		3 (21.4)	
	Multiple sibs	19 (2.0)		1 (5.3)	
	Other	2 (0.2)		1 (50.0)	
6. Gluten consumption by the mother during pregnancy					0.04
	No	509 (53.9)	944 (100)	61 (12.0)	
	Yes	435 (46.1)		74 (17.0)	
7. Mode of delivery					0.6
	Vaginally	398 (42.2)	569 (60.3)	57 (14.3)	
	C-section	171 (18.1)		27 (15.8)	
	Unknown	375 (39.7)		51 (13.6)	
8. Early intervention**					0.4
	Placebo	469 (49.7)	944 (100)	63 (13.4)	
	Gluten	475 (50.3)		72 (15.2)	

CD=celiac disease. C. Section= caesarean delivery. FDR= first degree relative. HLA= human leucocyte antigen. N.=number. Sib=sibling.

* data on the HLA risk group were available for 911 of 944 children with HLA typing performed by means of single-nucleotide polymorphisms (SNPs) on the basis of the tag-SNP approach. From 2 children who developed CD no HLA risk group was known; HLA risk groups: 1: DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); 2: DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); 3: DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); 4: DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); 5: DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); 'other': any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

** Early intervention consisted of 100 mg of gluten per day or placebo between 4-6 months of age (Vriezinga 2014).

cal settings in which the prediction models are meant to be used, model building was repeated without quantity of daily gluten intake. For baseline and landmark prediction models, risk scores were calculated by adding the regression coefficients from the multivariable Cox models. The risk scores were divided into low, low-medium, high-medium and high-risk groups and Kaplan-Meier estimates were calculated. Harrell's c-index was calculated to quantify discrimination of the resulting models.

Validation cohort

Validation analysis of the produced models was performed using data of the independent NeoCel cohort, in which all children were assessed regularly from birth for CD development at pre-defined intervals, in a similar way as in the PreventCD cohort (Supplementary Appendix).

The risk score as developed in the PreventCD cohort was calculated for every child in the NeoCel cohort. The children were subsequently allocated to one of the four risk groups. A univariate Cox model with the (continuous) risk score was fitted in the NeoCel cohort. Ideally, this should give a regression coefficient of 1; values significantly smaller than 1 indicate overfitting of the original risk score. Kaplan-Meier estimates were calculated for each of the four risk groups. Harrell's c-index was calculated to quantify discrimination.

RESULTS

PREVENTCD-COHORT

The mean age of the children (n= 944) was 10.3 years (range 8.4-12.0), 52% male, interquartile range (IQR) follow-up from 5.9 to 9.7 years. In total 227 (24%) children stopped participation (Supplementary Appendix). The distribution of the baseline variables of the cohort is presented in table 1.

Diagnosis of celiac disease

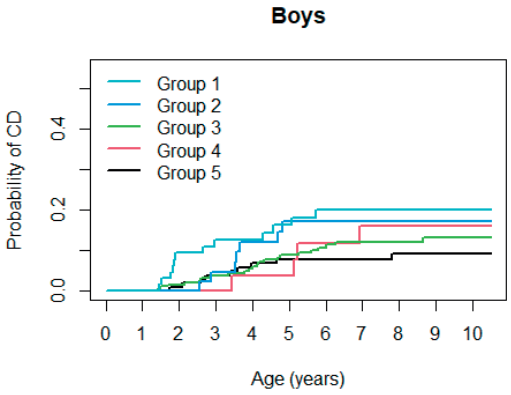
In total, 135 children were diagnosed with CD, including five without small-bowel biopsies according to the non-biopsy ESPGHAN criteria (Figure S1, Supplementary Appendix).[1] In total, 8363 TGA determinations were performed (Figure S2, Supplementary Appendix) with 563 children (59.6%) having at least one determination between March 2016 and March 2019. Mean age at diagnosis was 4.3 years (range 1.1-11.4). The cumulative incidence of CD was 7.5%, 16.6% and 17.5% at three, eight and ten years of age, respectively (Figure S3 Supplementary Appendix).

Variables related to CD development

CD developed significantly more frequently in girls (n=79, 59% vs n=56, 41%) (p=0.005) (Figure S4 Supplementary Appendix). Moreover, the frequency of CD development was significantly higher in children homozygous for HLA-DQ2 (DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2), than children with other HLA-DQ haplotypes, with a cumulative incidence at eight years of 35.4% (n =40) versus maximum 18.2% (HLA risk group 2, n = 14) (P<0.001) (Figure S5 Supplementary Appendix). This difference was even more significant when analysed separately for children with DR3-DQ2/DR3-DQ2 (n=21; 45.0%) compared to those with DR3-DQ2/DR7-DQ2 (n=19; 28.9%) (overall p<0.001) (Figure S6 Supplementary Appendix).

The interaction between gender and HLA risk group was not significant (p=0.10) with hazard ratios for HLA-DQ2 homozygous (DR3-DQ2/DR3-DQ2 and DR3-DQ2/DR7-DQ2) being 13.3 for girls (95% confidence interval [CI], 4.7-38.1; p<0.001)) and 2.4 for boys [95%CI, 1.0-5.7; p = 0.14) (Figures 1a and 1b).

Figure 1a. Cumulative Incidence of celiac disease in the PreventCD cohort (n = 911) at selected ages, according to five HLA-haplotype and male gender (n= 472).

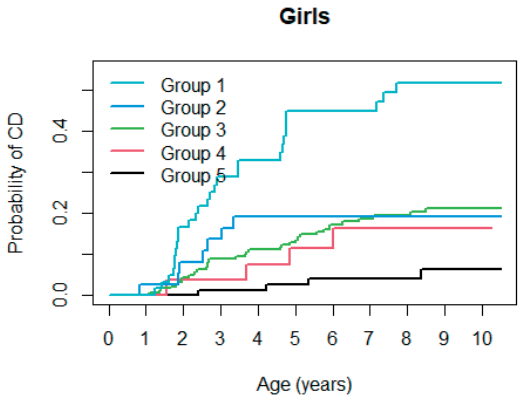


HLA risk	CD/N children at risk at ages					
	0 years	2 years	4 years	6 years	8 years	10 years
Group 1	0/67	6/57	2/48	4/40	0/31	0/10
Group 2	0/48	0/44	5/34	2/29	0/24	0/12
Group 3	0/208	3/190	8/165	8/137	2/107	1/38
Group 4	0/34	0/30	2/50	2/21	1/17	0/7
Group 5	0/115	1/106	6/93	1/82	1/60	0/20

Covariates		Coeff	Se (coef)	Multivariate hazard ratio	95% confidence interval	p-value
HLA risk group (ref: group 5)	Group 1	0.8799	0.4416	2.4108	1.01-5.73	0.14
	Group 2	0.6752	0.5040	1.9644	0.73-5.28	
	Group 3	0.3130	0.3957	1.3676	0.63-2.97	
	Group 4	0.4962	0.6014	1.6424	0.51-5.34	

In addition, in the exploratory analysis separating the HLA-DQ2 homozygosity in HLA DR3-DQ2/DR3-DQ2 from DR3-DQ2/DR7-DQ2, the interaction was significantly different with respect to gender (p=0.04). In girls, the risk to develop CD was significantly increased in both groups of HLA-DQ2 homozygosity, with hazard ratios of 14.8 [95%CI 4.8- 46.0] and 12.5 [95%CI 4.2-37.4] for DR3-DQ2/DR3-DQ2 and for DR3-DQ2/DR7-DQ2, respectively. In boys, the risk to develop CD was also significantly increased in those with DR3-DQ2/DR3-DQ2, but not in those with DR3-DQ2/DR7-DQ2 with hazard ratios of 5.0 (95%CI 2.0-12.6) and 1.0 (95%CI 0.3-3.5) respectively (Figures S7a and S7b Supplementary Appendix).

Figure 1b. Cumulative Incidence of celiac disease (CD) in the PreventCD cohort (n = 911) at selected ages, according to HLA-haplotype divided into five HLA groups and female gender (n = 439).



HLA risk	CD/N children at risk at ages					
	0 years	2 years	4 years	6 years	8 years	10 years
Group 1	0/62	10/49	6/52	9/33	3/20	0/4
Group 2	0/40	3/34	9/61	4/27	0/19	0/8
Group 3	0/209	8/181	21/320	13/155	3/97	2/38
Group 4	0/32	1/27	2/50	1/24	1/10	0/3
Group 5	0/96	0/83	7/166	1/73	0/53	1/12

Covariates		Coeff	Se (coef)	Multivariate hazard ratio	95% confidence interval	p-value
HLA risk group (ref: group 5)	Group 1	2.5903	0.5352	13.3342	4.67-38.07	<0.001
	Group 2	1.5156	0.6269	4.5521	1.33-15.55	
	Group 3	1.4424	0.5271	4.2310	1.51-11.89	
	Group 4	1.1180	0.7077	3.0587	0.76-12.25	

CD= Celiac disease, No=number, CI= confidence interval. Coeff: coefficient. HLA risk group; 1: DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); 2: DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); 3: DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); 4: DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and 5: DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); "other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

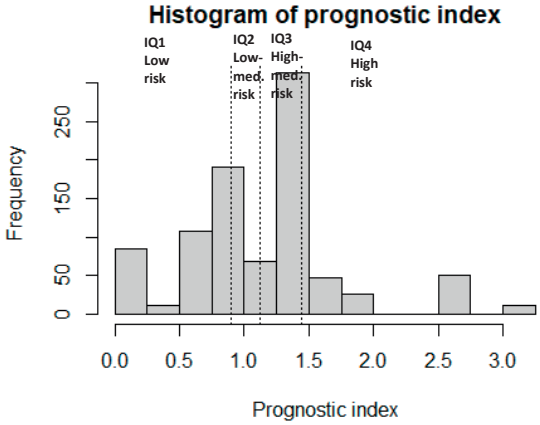
In multivariate analysis, no secondary variable, including early intervention with small quantities of gluten or breastfeeding, showed a significant association with CD development . In the landmark analyses, only a higher amount of average daily gluten intake during the first three years of age was associated with a higher risk to develop CD (p=0.07, p=0.03 and p=0.05 respectively) (Table S1 Supplementary Appendix). The prediction models built with and without the gluten intake per age showed similar results (Table 2).

Table 2. Hazard ratios for the prediction models with and without gluten consumption during the first three years of life in children from celiac families based on data from the PreventCD cohort (n=944)

Age (year)	One		Two		Three	
	With gluten consumption	Without gluten consumption	With gluten consumption	Without gluten consumption	With gluten consumption	Without gluten consumption
Gender (ref male)						
Female	1.65 (1.15-2.36)	1.64 (1.15-2.34)	1.48 (0.99-2.23)	1.47 (0.98-2.21)	1.27 (0.79-2.05)	1.26 (0.78-2.03)
HLA risk group (ref group 5)						
Group 1	6.70 (3.48-12.89)	5.95 (3.10-11.41)	4.68 (2.26-9.71)	3.97 (1.92-8.21)	4.33 (1.80-10.41)	3.60 (1.51-8.61)
Group 2	2.92 (1.31-6.51)	2.76 (1.24-6.15)	2.77 (1.17-6.52)	2.58 (1.10-6.07)	2.73 (0.99-7.54)	2.55 (0.92-7.03)
Group 3	2.51 (1.34-4.68)	2.34 (1.25-4.36)	2.28 (1.18-4.41)	2.07 (1.07-4.00)	2.34 (1.08-5.08)	2.12 (0.98-4.58)
Group 4	2.26 (0.92-5.53)	2.20 (0.90-5.38)	2.22 (0.86-5.73)	2.13 (0.83-5.50)	3.16 (1.14-8.72)	3.00 (1.09-8.30)
Number of FDR (ref 1)						
≥2	1.64 (1.01-2.67)	1.60 (0.99-2.59)	1.75 (1.00-3.05)	1.69 (0.97-2.94)	1.80 (0.94-3.45)	1.72 (0.90-3.31)
Gluten intake*						
Per gram intake	1.28 (1.09-1.50)	-	1.41 (1.15-1.72)	-	1.43 (1.13-1.82)	-

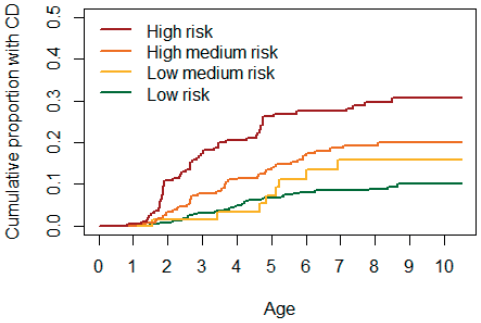
*up to a maximum of 5 grams gluten (see figure S9)

Figure 2a. Histogram of the prognostic index for development of celiac disease; 1. low risk: 0-0.90 points; 2. low-medium risk: 0.91-1.12 points; 3. high-medium risk: 1.13-1.44 points and 4. high risk: >1.45 points.



IQ= interquartile

Figure 2b. Cumulative incidences of celiac disease (CD) at different ages for the four risk groups.



	Risk groups PreventCD cohort			
	High (n)	**High-medium (n)**	**Low-medium (n)**	**Low (n)**
HLA risk group	193	255	68	395
1	129	0	0	0
2	44	44	0	0
3	15	211	61	0
4	5	0	61	0
5	0	0	7	204
CD	52	42	8	31

CD=celiac disease. N=number. HLA=human leucocyte antigen. HLA risk groups: group 1= DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); group 2= DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); group 3 = DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); group 4 = DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); and group 5= DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other);"other" refers to any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

Age (years)	High risk Events/at risk		High-medium risk Events/at risk		Low-medium risk Events/at risk		Low risk Events/at risk	
	Events /at risk	Cum. incidence (95% CI)	Events /at risk	Cum. Incidence (95% CI)	Events /at risk	Cum. incidence (95% CI)	Events /at risk	Cum. incidence (95% CI)
0.5	0/190	0	0/243	0	0/65	0	0/377	0
1	1/188	0.5 (0-1.5)	0/238	0	0/62	0	0/368	0
1.5	4/182	2.7 (0.3-4.9)	2/235	0.8 (0-2.0)	0/62	0	2/360	0.6 (0-1.3)
2	15/162	10.8 (6.2-15.2)	6/224	3.4 (1.1-5.7)	1/59	1.6 (0-4.8)	1/356	0.8 (0-1.8)
2.5	4/156	13.0 (8.0-17.8)	3/219	4.7 (2.0-7.4)	0/59	1.6 (0-4.8)	3/347	1.7 (0.3-3.0)
3	8/145	17.5 (11.8-22.8)	7/205	7.8 (4.2-11.1)	0/55	1.6 (0-4.8)	5/335	3.1 (1.3-4.9)
3.5	4/130	19.9 (13.9-25.6)	1/196	8.2 (4.6-11.7)	1/53	3.5 (0-8.1)	2/318	3.7 (1.7-5.6)
4	1/128	20.6 (14.4-26.3)	7/186	11.5 (7.2-15.6)	0/53	3.5 (0-8.1)	4/311	4.9 (2.6-7.2)
4.5	1/126	21.2 (14.9-27.0)	0/185	11.5 (7.2-15.6)	0/52	3.5 (0-8.1)	4/301	6.2 (3.6-8.7)
5	8/117	26.2 (19.3-32.5)	4/179	13.4 (8.8-17.8)	2/48	7.2 (0.1-13.8)	2/294	6.8 (4.1-9.4)
5.5	1/113	26.8 (19.9-33.1)	3/171	14.9 (10.1-19.5)	2/43	11.2 (2.3-19.4)	2/276	7.4 (4.6-10.2)
6	1/108	27.5 (20.5-33.9)	4/159	16.9 (11.8-21.8)	0/40	11.2 (2.3-19.4)	2/267	8.1 (5.1-11.0)
6.5	0/104	27.5 (20.5-33.9)	2/137	18.0 (12.7-23.0)	1/36	13.5 (3.6-22.3)	1/246	8.5 (5.4-11.4)
7	0/104	27.5 (20.5-33.9)	1/134	18.6 (13.2-23.7)	1/32	15.9 (5.0-25.6)	0/236	8.5 (5.4-11.4)
7.5	2/89	29.0 (21.8-35.5)	1/127	19.3 (13.7-24.5)	0/31	15.9 (5.0-25.6)	0/224	8.5 (5.4-11.4)
8	1/85	29.8 (22.5-36.4)	0/118	19.3 (13.7-24.5)	0/29	15.9 (5.0-25.6)	1/206	8.9 (5.7-12.0)
8.5	1/70	30.8 (23.3-37.5)	1/110	20.0 (14.3-25.3)	0/25	15.9 (5.0-25.6)	1/166	9.4 (6.1-12.7)
9	0/53	30.8 (23.3-37.5)	0/90	20.0 (14.3-25.3	0/20	15.9 (5.0-25.6)	1/130	10.0 (6.5-13.4)
9.5	0/37	30.8 (23.3-37.5)	0/65	20.0 (14.3-25.3	0/16	15.9 (5.0-25.6)	0/92	10.0 (6.5-13.4)
10	0/29	30.8 (23.3-37.5)	0/50	20.0 (14.3-25.3	0/8	15.9 (5.0-25.6)	0/65	10.0 (6.5-13.4)

CD= celiac disease; childr.=children; CI= confidence interval; HLA= Human Leukocyte Antigen; n=number of children

Prediction models

Based on the variables' regression coefficients in this multivariate model, a risk stratification score was constructed for each child (Table 3). Median (1.12) and first and third IQR (IQ1=0.90 and IQ3=1.44) were used as cut-off values for dividing the risk groups into low (0-0.90 points), low-medium (0.91-1.12 points), high-medium (1.13-1.44 points) and high (≥1.45 points) risk score (Figure 2a). The total points score is mapped as a corresponding risk of CD probability (Figure 2b).

Validation of the prediction model in the NeoCel cohort

The distribution of the variables in the NeoCel cohort contributing to the risk scores and probability for CD is presented in Table 4. Figure S8 (Supplementary Appendix) shows the estimated cumulative incidence of CD for each risk group in the NeoCel cohort. Cox regression with the continuous risk score yielded a regression coefficient of nearly 1.0 (0.81 (0.54); p=0.13), indicating good fitting despite the non-significance, with the

Table 3. Multivariate logistic regression model and corresponding risk score of probability of Celiac Disease development in children from celiac families based on data from the PreventCD cohort

	Hazard ratio	95% CI	p-value	Regr. coef./ Points for risk score at birth	Regr. coef./ Points for risk score at 1 year	Regr. coef./ Points for risk score at 2 year	Regr. coef./ Points for risk score at 3 year	Regr. coef./ Points for risk score at 4 year	Regr. coef./ Points for risk score at 5 year	Regr. coef./ Points for risk score at 6 year	Regr. coef./ Points for risk score at 7 year	Regr. coef./ Points for risk score at 8 year
Gender (ref male)												
Female	1.71	1.21-2.42	0.002	**0.54**	**0.49**	**0.38**	**0.23**	**0.38**	**0.47**	**0.85**	**1.42**	**1.09**
HLA risk group (ref group 5)												
Group 1	5.73	3.06-10.74	<0.001	**1.75**	**1.78**	**1.38**	**1.28**	**1.69**	**1.29**	**1.17**	**1.18**	**0**
Group 2	2.76	1.30-5.88	0.008	**1.02**	**1.02**	**0.95**	**0.94**	**0.17**	**0**	**0**	**0**	**0**
Group 3	2.25	1.23-4.10	0.008	**0.81**	**0.85**	**0.73**	**0.75**	**0.94**	**1.08**	**0.75**	**0.03**	**0.39**
Group 4	2.03	0.84-4.90	0.115	**0.71**	**0.79**	**0.76**	**1.10**	**1.25**	**1.61**	**1.40**	**0**	**0**
Number of FDR (ref 1)												
≥2	1.51	0.93-2.44	0.09	**0.41**	**0.47**	**0.52**	**0.54**	**0.62**	**0.57**	**0.67**	**0.71**	**2.1**

CI= confidence interval. FDR=first degree relative. HLA= Human Leukocyte Antigen. ref=reference

Table 4. Distribution of the variables in the Neocel cohort contributing to the risk scores and prediction models for CD (n=162)

Variable	Values	N (%)	Total (%)	CD (%)
1. Gender	Male	79 (48.8)	162 (100)	6 (7.6)
	Female	83 (51.2)		7 (8.4)
2. HLA risk group*	Group 1	3 (2.6)	117 (72.2)	1 (33.3)
	Group 2	13 (11.1)		4 (30.8)
	Group 3	13 (11.1)		0 (0)
	Group 4	54 (46.2)		5 (9.3)
	Group 5	34 (29.1)		2 (5.9)
3. Number of affected FDR	1	137 (84.6)	162 (100)	13 (9.5)
	2 or more	12 (7.4)		0 (0)
4. Risk score groups	High	19 (16.3)	117 (100)	4 (21.1)
	High-medium	12 (10.3)		1 (8.3)
	Low-medium	49 (41.2)		5 (10.2)
	Low	37 (31.6)		2 (5.4)

CD=celiac disease. FDR= first degree relative. HLA= human leucocyte antigen. N=number; * HLA risk group known in 117/162 children (n 1 child who developed CD was not HLA typed). Groups 1: DR3–DQ2/DR3–DQ2 (DQ2.5/DQ2.5) and DR3–DQ2/DR7–DQ2 (DQ2.5/DQ2.2); 2: DR7–DQ2/DR5–DQ7 (DQ2.2/DQ7); 3: DR3–DQ2/DR5–DQ7 (DQ2.5/DQ7), DR3–DQ2/DR4–DQ8 (DQ2.5/DQ8), and DR3–DQ2/other (DQ2.5/other); 4:DR7–DQ2/DR7–DQ2 (DQ2.2/DQ2.2), DR7–DQ2/DR4–DQ8 (DQ2.2/DQ8), and DR4–DQ8/DR4–DQ8 (DQ8/DQ8); 5: DR7–DQ2/other (DQ2.2/other), DR4–DQ8/DR5–DQ7 (DQ8/DQ7), and DR4–DQ8/other (DQ8/other); 'other': any HLA-DQ haplotype except DR3–DQ2, DR7–DQ2, DR4–DQ8, or DR5–DQ7.

risk scores based on the data of the PreventCD cohort. The Harrell's c-index of 0.608, somewhat smaller than in the PreventCD cohort, is not surprising, considering the contribution of the factors could be estimated to optimize discrimination in the original PreventCD cohort.

DISCUSSION

Although long term follow-up cohorts of children genetically predisposed for CD have been reported before[11], we here present the longest follow up data from a birth cohort of genetically predisposed children with FDR with CD. Based on this prospective data, we developed prediction models for CD development in children from CD families to facilitate their individualized screening advice for CD.

Our results show first that the risk to develop CD for children with affected FDR during the first ten years of life is significantly higher than previously assumed.[6] Until recently, the lifetime risk of CD for FDR of CD patients was considered to be 5%-10%, yet our data show that at the age of eight years, this is as high as 17%, emphasizing the importance

of a sound advice for early screening.[6,12-17] We also confirm that CD develops in children with affected FDR at a very young age, as the mean age of diagnosis in our cohort was four years of age. This early development has also been shown in screening studies among the general pediatric population, and we can assume that, in general, this can be accepted as part of the natural history of CD.[18-23] We additionally confirm that, as previously reported by us in the same cohort at the age of three years, the risk of CD in these children during their first 10 years of life is strongly related to their gender and HLA-DQ phenotype.[5] In total, at the age ten years, girls have a 7.7% higher cumulative incidence compared to boys (21.5% vs 13.8%). The increased risk for CD in HLA-DQ2 homozygotes as well as the predominance of female gender is well known.[7,8] However, the significant additional effect of the interaction between female gender and certain HLA-DQ2 homozygosity has not been reported before. Contrary to HLA-DR3-DQ2 homozygosity, the increased risk in HLA-DR3-DQ2/DR7-DQ2 homozygosity is only present in females. This different effect of gender appears very early in life, and it persists and increases during the first ten years of age (cumulative incidence 8.0% for boys and 51.3% for girls) (Figure S7a and 7b Supplementary Appendix). The reason for this difference is unknown and intriguing and possible explanations are offered in the Supplementary Appendix.

In contrast to previously reported results by our group, the present results show that the quantity of early gluten intake is associated with a significantly higher risk of CD development, with an increased hazard ratio of 1.07 per gram increase in daily gluten intake.[24] Plausible explanations for the discrepancy are the different statistical methods used to analyse the data, since we now have used landmark analyses to avoid immortal time bias.[24] Since the prediction models with and without adding the amount of gluten intake per age show similar results, we have chosen to use the models without gluten intake, as this is generally unknown in standard clinical setting. Our present findings are in accordance with those from the TEDDY and DAISY studies,[25,26] suggesting that the quantity of gluten ingestion may be a preventive factor for CD. Indeed, the plots of the average daily gluten intake by the children in our study suggest that the risk of CD increases linearly until approximately 5 grams per day, and that more gluten consumption per day does not longer increases the risk of CD (Table 2 and Figure S9, Supplementary Appendix). However, it is important to keep in mind that these data are observational and no causality may be concluded. These observations do not allow us (or others) at this moment to give recommendation to the parents on the prevention of CD in their children. To develop such recommendations the results of RCTs with different quantities of ingested gluten as intervention are needed.

Screening advice

Screening for CD is recommended in children with FDR with this condition, but the frequency of screening and at what age remains unknown.[27,28] Based on our prediction models of CD, an individualized screening advice for children with FDR with CD can be provided (Figure 2b). Children in the high-risk group should be advised to start screening for CD earlier in life and more often than children in other risk groups. This also depends upon the current age of the child, since the risk of CD changes accordingly (Table 3). To calculate the child-tailored risk and give a personalized screening advice, we designed a Prediction application (https://hputter.shinyapps.io/preventcd/) based on both the risk group to which the child belongs and the current age of the child. As basis for our advice, we use the current standard of care of many centres taking care of families with CD, which is comprised of a yearly screening of children with FDR with CD based on the assumption of a 10% cumulative incidence among them. As a result, we advise that every child with a FDR with CD should be screened at presentation, including total IgA and IgA-TGA determination, as well as HLA-DQ2 and DQ8 typing. If the results of the TGA are negative, the risk of developing CD in the next years should be assessed using our Prediction app. If the prediction for CD development is higher than 10% in the next two years, we advise to repeat the screening after six months. If the prediction is between 5-10%, the advice is to repeat the screening after one year and if the prediction is lower than 5%, to repeat the screening after two years. For example, if we assume the case of a 1-year-old girl HLA-DR3-DQ2 homozygous with normal IgA and negative TGA, we will advise her to repeat the screening at 18 months and two years of age (prediction 18.9% in the next two years). For more examples concerning the use of the Prediction app for screening advice, see the Supplementary Appendix.

The strength of our models for CD development and screening advice is that they are based on prospective data from multicentre collaboration with a long follow-up time. All children have been followed in a homogenous manner, with centralized TGA determinations (nine of the ten centres) and assessment of diagnostic biopsies, thereby minimizing the risk of diagnostic bias. The high number of CD-diagnosed cases in our cohort benefits also the design of the prediction model. The multicentre, multinational involvement in the PreventCD cohort, and therefore the plausible influence of different environmental factors in the results and consequently in the produced prediction model, make it applicable in different countries. Lastly, the validation of the prediction model in an external independent high-risk CD cohort with good fitting, supports the implementation to improve medical care and continuously optimize the model. Although individualized screening advice for CD has been reported before,[26] as far as we know, we are the first to provide it including age of initiation and frequency of screen-

ing, in the form of a clinically easy-to-use Prediction app (https://hputter.shinyapps.io/preventcd/).

Possible shortcomings of our study are the variable intervals of TGA determination after the age of three years, implying that the CD development may occur sometime prior to TGA determination. We have taken this into account by averaging the time of CD development between the last negative TGA result and the date of CD diagnosis. Another possible shortcoming is that TGA determination was done in 563/944 children during the last three years of follow up (59.6%).From the 154 children who had no TGA determination during the last three years, we have negative TGA results till a mean age of 5.1 years (3.0 - 8.2 years). However, from the 167 children whose parents withdrawn consent for the study we have negative TGA results till a mean age of 3.2 years (3 months - 9.4 years) and we have included all these data to develop the prediction models and application (see the Supplementary Appendix). Taking all this into consideration, our nearly 60% follow-up rate after 10 years can be considered as quite acceptable.

We have analysed data till the age of 10 years, and our prediction application applies till the age of 8 years. This is inherent to the data available at the time at which the data was frozen for analysis, when all the participants had reached the age of 8 year (range 8.4-12.0). It should be noted that this Prediction app and screening advice have been developed for children from CD families and should therefore not be applied in children from the general population until their use has been broadly validated.

To conclude, children with CD-FDR develop CD early in life, and their risk depends on gender, age and HLA-DQ: all factors which are important for a sound screening advice. These children should be screened early in life, including HLA-DQ2/8 typing, and if genetically predisposed to CD, should get a further personalized screening advice using our Prediction app (https://hputter.shinyapps.io/preventcd/).

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of celiac disease. J Pediatr Gastroenterol Nutr 2012;54(1):136-60.
2. Vriezinga SL, Schweizer JJ, Koning F, Mearin ML. Celiac disease and gluten-related disorders in childhood. Nat Rev Gastroenterol Hepatol. 2015 Sep;12(9):527-36
3. Lindfors K, Ciacci C, Kurppa K, et al. Celiac Disease. Nat Rev Dis Primers. 2019 Jan 10;5(1):3. doi: 10.1038/s41572-018-0054-z.
4. Lionetti E, Castellaneta S, Pulvirenti A, et al; Italian Working Group of Weaning and Celiac Disease Risk. Prevalence and natural history of potential celiac disease in at-family-risk infants prospectively investigated from birth. J Pediatr. 2012 Nov;161(5):908-14.
5. Vriezinga SL, Auricchio R, Bravi E, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med 2014; 2;371(14):1304-15.
6. Singh P, Arora S, Lal S, Strand TA, Makharia GK. Risk of celiac disease in the first- and second-degree relatives of patients with celiac disease: a systematic review and meta- analysis. Am. J. Gastroenterol. 2015, 110, 1539–1548.
7. Liu E, Lee HS, Aronsson CA, et al; TEDDY Study Group. Risk of pediatric celiac disease according to HLA haplotype and country. N Engl J Med. 2014 Jul 3;371(1):42-9.
8. Margaritte-Jeannin P, Babron MC, Bourgey M, et al. HLA-DQ relative risks for celiac disease in European populations: a study of the European Genetics Cluster on Celiac Disease. Tissue Antigens. 2004;63:562–567
9. Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control, 19 (6): 716–723, doi:10.1109/TAC.1974.1100705.
10. Hastie T, Tibshirani R, Friedman J. The Elements of Statistical Learning: Data Mining, Inference, and Prediction. Second Edition. Springer, New York, 2009
11. Liu E, Dong F, Barón AE, Taki I, Norris JM, Frohnert BI, et al. High Incidence of Celiac Disease in a Long-term Study of Adolescents With Susceptibility Genotypes. Gastroenterology 2017 May;152(6):1329-1336
12. Oliveira A, Trindade E, Tavares M, Lima M, Dias JA. Celiac disease in first degree relatives of celiac children. Arg Gastroenterol. 2012; 49(3):204-7.
13. Dogan Y, Yildirmaz S, Ozercan IH. Prevalence of celiac disease among first-degree relatives of patients with celiac disease. J Pediatr Gastroenterol Nutr. 2012; 55(2):205-8.
14. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 2003; 163(3):286-92.
15. Almeida PL, Gandolfi L, Modelli IC, Cássia Martins R, Coutinho de Almeida R, Pratesi R. Prevalence of celiac disease among first degree relatives of Brazilian celiac patients. Arg Gastrenterol. 2008; 45(1):69-72.
16. Pittschieler K, Gentili L, Niederhofer H. Onset of celiac disease: a prospective longitudinal study. Acta Pediatr 2003;92(10):1149-1152.
17. Mäki M, Mustalahti K, Kokkonen J, et al. Prevalence of celiac disease among children in Finland. N. Engl. J. Med. 2003 Jun 19;348(25):2517-24. doi: 10.1056/NEJMoa021687.
18. Mustalahti K, Catassi C, Reunanen A, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med. 2010 Dec;42(8):587-95. doi: 10.3109/07853890.2010.505931.

19. Bingley PJ, Williams AJK, Norcross AJ, et al. Undiagnosed celiac disease at age seven: population based prospective birth cohort study. BMJ 2004 Feb 7;328(7435):322-3. doi: 10.1136/bmj.328.7435.322.
20. Katz KD, Rashtak S, Lahr BD, et al. Screening for celiac disease in a North American population: sequential serology and gastrointestinal symptoms. Am J Gastroenterol. 2011 Jul;106(7):1333-9. doi: 10.1038/ajg.2011.21.
21. Hovell CJ, Collett JA, Vautier G, et al. High prevalence of celiac disease in a population-based study from Western Australia: a case for screening? Med J Aust. 2001 Sep 3;175(5):247-50.
22. Jansen M, van Zelm M, Groeneweg M, et al (2018) The identification of celiac disease in asymptomatic children: the Generation R Study. J Gastroenterol. 2018 Mar;53(3):377-386. doi: 10.1007/s00535-017-1354-x53(3):377-386.
23. Catassi C, Kryszak D, Louis-Jacques O, et al. Detection of Celiac disease in primary care: a multicenter case-finding study in North America. Am J Gastroenterol. 2007 Jul;102(7):1454-60. doi: 10.1111/j.1572-0241.2007.01173.x.
24. Crespo-Escobar P, Mearin ML, Hervás D, et al. The role of gluten consumption at an early age in celiac disease development: a further análisis of the prospective PreventCD cohort study. Am J Clin Nutr. 2017 Apr;105(4):890-896.
25. Aronsson CA, Lee HS, Koletzko S, et al. TEDDY Study Group. Effects of Gluten Intake on Risk of Celiac Disease: A Case-Control Study on a Swedish Birth Cohort. Clin Gastroenterol Hepatol. 2016 Mar;14(3):403-409.
26. Mårild K, Dong F, Lund-Blix NA, et al. Gluten Intake and Risk of Celiac Disease: Long-Term Follow-up of an At-Risk Birth Cohort. Am J Gastroenterol. 2019 Aug;114(8):1307-1314.
27. Bai J, Ciacci C. The World Gastroenterology Organisation Global Guidelines recommend testing for CD in asymptomatic children who have first-degree relatives with the disease. J Clin Gastroenterol. Volume 51, number 9. Febr 2017.
28. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Pediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Celiac Disease 2020. J Pediatr Gastroenterol Nutr. 2020 Jan;70(1):141-156.
29. Wessels MMS, de Rooij N, Roovers L, Verhage J, de Vries W, Mearin ML. Towards an individual screening strategy for first-degree relatives of celiac patients. Eur J Pediatr. 2018 Nov;177(11):1585-1592.

6

Association in Clinical Practice between Gluten-Intake and Detection of Gluten Immunogenic Peptides in Celiac Children

Gastro Hep Advances 2022; 1:652–65

Caroline R. Meijer
Jaap Bakker
Anneloes Boers
Sophie Jansen
Zeliha Mengi
M. Luisa Mearin

ABSTRACT

BACKGROUND AND AIM: The dietary compliance and its assessment in celiac disease (CD) patients on a strict gluten-free diet (GFD) remain a challenge. Two relatively new, validated methods have been proposed to detect occasional gluten ingestion: standardized dietary questionnaire and determination of urinary gluten immunogenic peptides (GIP). Our aim was to prospectively assess dietary compliance via these methods and compare their results with those of tissue-transglutaminase antibodies (TGA).

METHODS: Prospective single-centre. Consecutive CD-patients (1-18 years) on a GFD scheduled for regular consultation between March-August 2019 were invited. In addition to standard care, a completed dietary questionnaire and urine sample for GIP were collected. Pearson's chi square test, Fisher's exact test and Mann-Whitney U test were performed.

RESULTS: 110 of the 156 eligible children provided informed consent. Completed dietary questionnaire-, GIP- and TGA-results were available from 86 children (median age 12.8 years, median GFD-duration 30 months, 65% female). Adherence to the GFD evaluated by GIP, dietary questionnaire and anti-TGA was 94.2%, 75.6% and 94.2% respectively. No association was found between the TGA results and the detection of GIP as well as between the TGA results and the dietary questionnaires scores ($p = 0.5$ and 0.312 respectively). The participants perceived both the questionnaire and the measurement of GIP as reassuring with regards to correct implementation of the GFD.

CONCLUSION: All the three methods have limitations to monitor dietary compliance. The comparison of their performance shows that the best, single method is the use of the validated dietary questionnaire which should therefore be implemented in the regular care for children with CD. The most effective combination of dietary questionnaire and urinary GIP determination should be used in specific clinical situations.

INTRODUCTION

The only effective treatment for celiac disease (CD) is a strict life-long gluten-free diet (GFD) which usually improves symptoms, restores small bowel histology and avoids long-term complications[1,2]. Nevertheless, dietary adherence is a challenge due to dietary restrictions, poor labelling regulations, sociocultural restrictions, decreased QoL and limited availability and high costs of gluten-free alternatives[3-5]. Dietary compliance in children with CD has been estimated as 25% - 50%[1,5,6]. The golden standard to assess mucosal healing (that is endoscopy and small bowel biopsies) is an invasive procedure that is not performed during regular check-ups. Usually, compliance with the GFD is evaluated by dietary interview with a dietician and/or by serum determination of IgA against tissue transglutaminase (TGA) [4,7]. However, both methods have limitations as TGA testing is insufficiently sensitive for detecting occasional dietary transgressions, and dietary evaluation by a trained dietician is time-consuming and not always available in clinical settings and dependent on patient-reporting [5,8,9].

Other methods to determine gluten ingestion by CD children are a standardized dietary questionnaire reflecting an regular interview by a specialized dietician and the measurement of gluten immunogenic peptides (GIP) in urine or stool [5,6,10,11]. GIP are small fragments of gluten resistant to gastrointestinal digestion causing the immunotoxic T-cells reaction in CD patients. A fraction of the GIP makes it into the circulation and is excreted in urine, being detectable after 4-6 hours and remain detectable for up to 24-36 hours[11]. The test is highly sensitive with a limit of detection of ≥ 50 mg of ingested gluten, taking into account that the maximal gluten ingestion during a strict GFD should not exceed 10-20 mg/day [12].

The aim of this study is to prospectively compare the performance of three methods to assess dietary compliance in clinical practice during the follow-up of CD children: via validated dietary questionnaire, GIP in urine and TGA determination in serum.

METHODS

Study population

For this prospective single-centre implementation study, consecutive patients with CD (1-18 years) attending the celiac out-patient clinic of the Leiden University Medical Centre (LUMC) for a regular follow-up visit, were recruited between March 2019 and August 2019. Inclusion criteria were: CD diagnosed according to the guidelines of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), following a GFD, parents/child having sufficient knowledge of the Dutch language and

written informed consent[13]. Consent was provided by parent/legal guardians and also by children ≥12years old. Patient characteristics were collected from the electronic medical record. All authors had access to the study data and reviewed and approved the final manuscript.

Procedure

Invitation to participate in the study was sent by letter to (parents of) the children 2-3 weeks prior to their appointment at the out-patient clinic. It was explained that participation included providing a urine sample for the detection of GIP collected at the day of the consultation. In addition, (parents of) children were asked to complete the Dutch version of the validated dietary questionnaire on the compliance with the GFD [5; Annex 1]. The questionnaire addresses several domains, including compliance with and knowledge of the GFD and the attitude towards the diet. Each answer corresponds with a point score, which were not visible for the (parents of) the children, providing a score between 0-84 which corresponds to: 1. Strict GFD (0-2 points); 2. GFD with important errors (3-20 points); and 3. GFD not followed (21-84 points)[5]. Furthermore, the children received the standard care for CD, including TGA determination (Thermofisher, Germany; ImmunoCAP250; cut-off of normality 7 U/ml).

GIP in urine was determined at the clinical chemistry laboratory of the LUMC, blinded for clinical information and TGA results, using the iVYCHECK GIP Urine kit (Biomedal, Spain), following the manufacturer instructions. The results were expressed as ng GIP per 1 mL of urine, with the limit of detection being 2.2 ng GIP/mL (>50 mg of ingested gluten). If dietary adherence was considered as insufficient by raised tGA titers, positive GIP or dietary questionnaire, a referral to a dietician was offered.

Statistical analysis

A Shapiro-Wilk-test was used to test for normality of the data. Where applicable, Pearson's chi-square test, Fisher's exact test or Mann-Whitney U test were used for evaluating baseline characteristics. Furthermore, these tests were used to estimate the strength of association between the outcomes reported in the dietary questionnaire, GIP and TGA levels. A two-tailed probability of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 25.0.

Ethical considerations

The study was approved by the Medical Ethics Committee of Leiden-Den Haag-Delft (P18.241).

RESULTS

The flowchart of the eligible children for the study is presented in figure 1. In total, 156 children were eligible for participation and (the parents of) 110 gave informed consent (70.5%): median age: 12.8 years, 71 females (64.5%). Characteristics of the children who gave informed consent and of the children who refused participation were similar, except for age above 13 years, since these children refused participation significantly more (p = 0.03, Table 1). In total 86 children had complete data (filled out dietary questionnaire and measured TGA and GIP) and were included in the analyses (Fig 1).

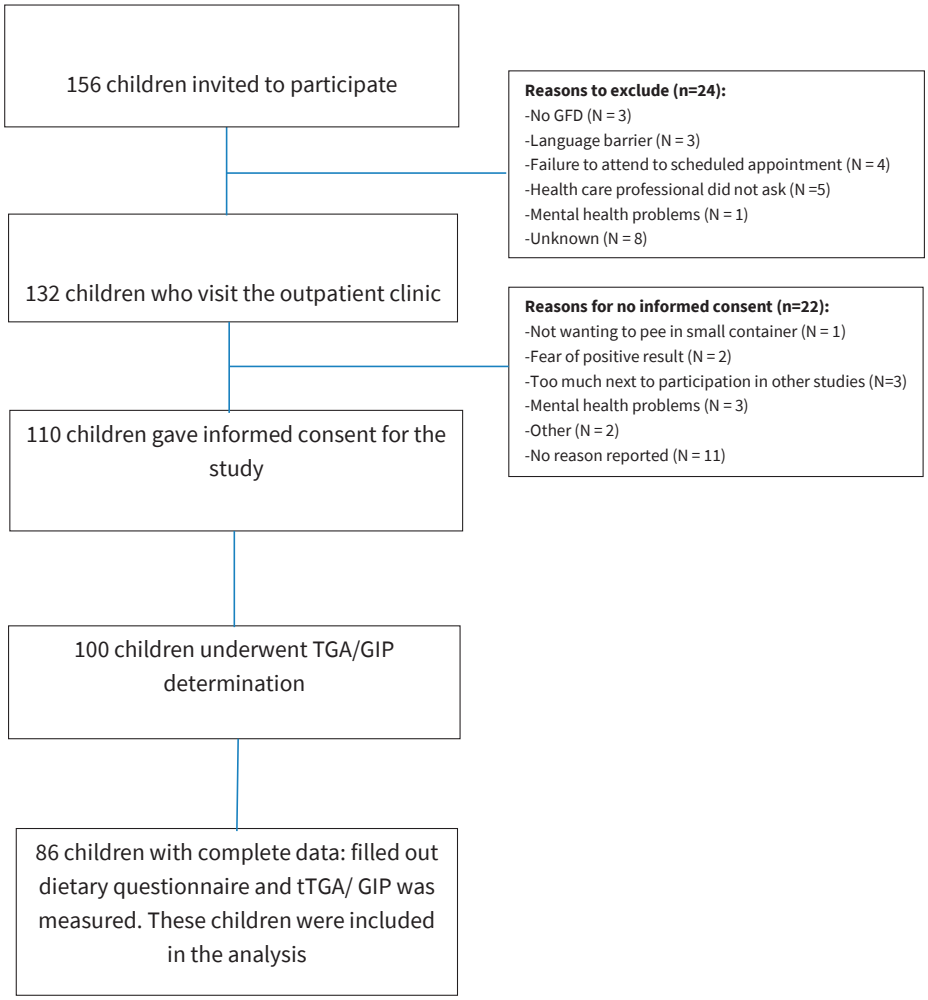


Figure 1. flowchart of the eligible children with celiac disease (CD), including the reasons for exclusion and no informed consent.

Table 1. Characteristics of the 110 children who gave informed consent for the study and the children who declined informed consent (n=22).

	Participating CD children (N = 110)	Declined informed consent (N = 22)	P-value
Age (years), median	12.8	11.1	0.128
Age groups, no. (%)			
0-4 years	16 (14%)	4 (18.2%)	0.06
5-12 years	61 (56%)	6 (27.3%)	0.13
≥ 13 years	33 (30%)	12 (54.5%)	**0.03**
Female, no. (%)	71(64.5%)	13 (59.1%)	0.1
Age at diagnosis of CD (years), median	4	7.1	0.158
Duration of GFD (months), median	30	74	0.24
0 – 24 months, no. (%)	35 (32%)	6 (27.2%)	0.31
25 – 48 months, no. (%)	21 (19%)	3 (13.6%)	0.83
≥ 49 months, no. (%)	54 (49%)	13 (59.1%)	0.68
Positive family history of CD, no. (%)	55 (50%)^	7 (31.8%)	0.318
Filled in dietary questionnaires, no. (%)	95 (86.3%)	-	
Score dietary questionnaire, mean (range)	1.77 (0 – 14)	-	
Anti-tTG measured, median (range)	8.9 (0-104)	7.7(0.1-44)	0.655
Elevated anti-tTG*, no. (% of measured anti-tTG)	22 (20%)	5 (22.7%)	0.922
GIP measured in urine, no. (%)	100 (90.1%)	-	
GIP present, no. (% of measured GIP)	5 (4.5%)	-	
GIP levels (ng/mL), median (range) ⍰	8.74 (7.88 – 14.27)	-	

CD=celiac disease, GFD=gluten free diet; GIP= gluten Immunogenic Peptides; tTG=anti-tissue transglutaminase type 2 antibodies; ^ Family history was unknown in 4 children # Score ≥ 3 on dietary questionnaire * Cut-off of normality > 7 U/mL ° Concentrations of > 128 U/mL were considered as a concentration of 128 U/mL, some titers were diluted for standard CD care giving a value above 128. ⍰ Median concentration from positive tests only

Twenty-one children (24.4%) had elevated TGA levels (median age 11.0 years (range 2-15)), median duration GFD 22.9 months (range 3-135). Of them, 16 had a decreasing trend in antibody-titers from the start of their GFD, with 10 children following the diet for less than 1 year. Increased TGA results were observed in 5 children, 4 of which were older than 11 years of age, with a median duration of GFD of 35 months (range 23-138). The children with elevated TGA (n=21) followed a GFD significantly shorter than those with negative antibodies (median duration 22.9 vs. 69.3 months; p = 0.02), although median age of both groups was similar (p = 0.937).

Five children (5.8%; 4 females; median age 8 years (range: 4-16)) had detectable urinary GIP with a median level of 8.74 ng/mL (7.88 – 14.27). Their characteristics were similar in terms of age, median duration of the GFD, age at diagnosis and gender to the ones of the GIP negative children (p = 0.382, p = 0.293, p = 0.996 and p = 0.068, respectively; data not shown).

Only the parents of a 7-year-old girl with positive GIP and rising TGA, who followed the GFD for 36 months, agreed to have a consultation with the dietician. The parents of the other 4 children reported 'not seeing an added value in an appointment with a dietician' as they already knew what caused the positive GIP test, including "gluten contamination or a small mistake at a new school", "probable mistake in hand hygiene", "probable mistake at grandparents or school" and "occasional intentional gluten consumption".

On the dietary questionnaire, 21 children (24.4%) reported important errors (median age 11.3 years (range 2-17; median duration GFD 64.4 months (range 4-193). The most frequently errors were "consuming food with a label "may contain traces of gluten or wheat" (n=42), "consuming food with a label "prepared in an environment where gluten/wheat is processed" (n=29) and "consuming naturally gluten-free flour with no gluten-free label or logo" (n=16). The characteristics of the children who reported strictly complied with the GFD (n=65) were similar to those reporting dietary errors (n=21) (Table 2).

Table 2. Comparison of the patient characteristics following a strict gluten-free diet or reporting dietary errors according to the dietary questionnaires

Score dietary questionnaire	Strict GFD* N = 65 (%)	GFD with important errors** N = 21 (%)	p-value
Median age, years	9.23	11.3	0.974
Age groups (years)			0.097
0-4	9 (13.8)	1 (4.7)	0.08
5-12	43 (66.2)	10 (47.6)	**0.01**
≥ 13	13 (20.0)	10 (47.6)	**0.01**
Median age at diagnosis (years)	4.97	5.29	0.398
Female	43 (68.3)	14 (60.9)	0.932
Sympt. after unintentional gluten intake	51 (81.0)	18 (78.2)	0.313
Positive family history for CD	34 (54.0)	9 (39.1)	0.467
Only one person following a GFD at home	37 (58.7)	8 (34.7)	0.374
Other dietary restrictions	6 (9.5)	1 (4.3)	0.184
Median duration of GFD in months (range)	38.7 (2-184)	64.4 (4-193)	0.533

CD= celiac disease; DQ=dietary questionnaire; GFD=gluten free diet; *strict GFD is defined as score between 0-2 ** scores between 3-20 on dietary questionnaire on adherence to the gluten-free diet [5]

In total 80 (93.0%) children and/or their parents stated that they had enough knowledge of the GFD. The 6 children/parents reporting insufficient knowledge, followed the GFD significantly shorter than the others (14 months vs 59 months; p=0.008). Two of these children had a positive urinary GIP and scored important errors on the dietary questionnaire.

The absence of association between the TGA results and the detection of urinary GIP as well as between the TGA results and the ones from the dietary questionnaires is presented in Table 3 (p = 0.5 and 0.312 respectively). Likewise, no significant association was found between the scores of the dietary questionnaire and measurement of GIP in the urine (p = 0.08) (Table 4).

Table 3. Association between TGA and GIP results and between TGA and dietary questionnaire (DQ) scores in 86 children

TGA n (%)	GIP n (%)		p-value	Score DQ			p-value
	Positive N=5	Negative N=81		Strict GFD N=65	Errors GFD N=21	Non adherence	
Elevated 21 (24)	2 (9)	19 (91)	0.5	17 (81)	4 (19)	0	0.312
Normal 65 (76)	3 (5)	62 (95)		48 (74)	17(26)	0	

Table 4. Association of GIP results and dietary questionnaire (DQ) scores in 86 children

Score DQ / GIP	Strict GFD N=65	Errors GFD N=21	p-value
Positive (N=5)	3	2	0.08
Negative (N=81)	62	19	

TGA= Anti-tissue transglutaminase antibodies GIP=gluten immunogenic peptides DQ=dietary questionnaire; GFD=gluten free diet; *strict GFD is defined as score between 0-2, ** important errors between 3-20 and *** not following the gluten-free diet on the dietary questionnaire[5]

DISCUSSION

To the best of our knowledge, this is the first study comparing two relatively new methods to detect (un)intentional non-compliance with the GFD in children with CD, namely a validated dietary questionnaire reflecting a regular dietary interview as performed by an experienced dietician and the measurement of urinary GIP. Our results did not show an association between the results of TGA and the dietary questionnaire, TGA and GIP or the dietary questionnaire and urinary GIP.

By using the dietary questionnaire, we found that 24.4% of our population was not fully compliant to the GFD versus 5.8% as assessed by urinary GIP. Elevated but decreasing TGA was found in 16 out of 21 children (76%) who followed the GFD for a relatively short time of 22.9 months, which is in agreement with the time-frame in which normalisation of TGA usually occurs[7,14,15]. Also, five children showed an increase/stagnation in their TGA titers (5.8%), but only one of them had positive GIP. Our results therefore show that the dietary questionnaire is the best single method to detect occasional gluten intake. The discrepancy between the dietary compliance assessed by the questionnaire and the results of TGA in our study confirms the lack of sensitivity of CD serology to detect occa-

sional transgressions as shown in previous studies[6,10,16-19]. Nevertheless, three out of five children with detectable GIP in their urine did not report dietary transgressions in their questionnaire. This suggests that the combination of the dietary questionnaire and urinary GIP is the most effective method to detect occasional/inadvertent gluten intake. To calculate the diagnostic performance of the evaluated methods in terms of sensitivity, specificity, PPV and NPV is not possible by lacking a gold standard to assess dietary compliance.

The non-compliance with the GFD of almost 25% found in our study using the questionnaire agrees with the previously reported non-compliance of 25%- 50%[5], indicating that our population is representative for children with CD. However, the number of patients with detectable GIP in their urine in our study (5.8%) is surprisingly low compared to other studies performed in CD children in other countries. A systematic review of the literature reported fecal GIP detection in 25% of the children[1]. Four prospective studies among children (two combined with adults) assessing diet adherence by fecal/urinary GIP showed non-compliance in 45%, 29.8%, 16% and 14.5% of patients, respectively[8,16,20,21]. This discrepancy may be explained by the difference in methodology. Our population received information two weeks prior to the consultation describing the aim of the study and the purpose of the use of the urine for detection of excreted gluten peptides. This may have established a time-frame in which the children could re-evaluate and improve their compliance with the diet, Also, urinary GIP as assessed in this study is only detectable for 36 hours after gluten ingestion, in comparison to 4-7 days in the faeces as used in other studies[8,20,21], and we may have missed dietary transgressions made before this time-frame. Another possible limitation of our study is the relatively small number of participating children, although it is comparable to (or even higher) than sample sizes from previous studies performed in children[8,11,16,21].

Most of the previous studies on GIP in faeces and/or urine did not describe the manner in which the included patients were informed[11,16,22]. It is possible that recruitment of patients on short notice could have led to a higher number of positive GIP in those studies, as the 16% reported by one study in which the participants were not specifically aware of the GIP-measurement [8]. Nevertheless, if urinary GIP determination are implemented in the regular consultations on the long-term the CD patients would become aware of it. As such, sending study-information prior to the consultation in our study is comparable with the possible implementation of GIP in the standard of care for CD children.

Another possible explanation of the low frequency of positive GIP in our study may be the high number of children older than 13 years who refused participation in the

age-category with the highest percentage of non-compliance to the GFD [16]. A possible reason for declined consent may have been the fear of the exposure of potential non-compliance with the diet through a positive GIP result.

In addition to detect errors in the GFD, urinary GIP determinations may also be used to guarantee or reassure (parents of) patients that the GFD is correctly adhered to. This was also reported in the interviews which were taken by a randomly selected number of children and/or parents after terminating the study. The majority of (parents of) the patients believed that the test had an added value, especially in children who remained symptomatic or who were still familiarizing themselves with the diet (results not shown).

Our results show that from the three evaluated methods, the dietary questionnaire is the best single one to detect non-compliance with the GFD compared to the other methods and we, therefore, propose it for assessing diet adherence during the regular follow-up of CD children. In addition, the validated dietary questionnaire also identifies sources of non-compliance, facilitating self-correction by the patient. With the increasing use of E-health, partly due to the COVID pandemic, completing and processing the questionnaire will become easier and the implementation in standard healthcare more accessible and less time-consuming.

The combination of the dietary questionnaire and urinary GIP test is the most effective method in detecting (un)intentional gluten transgressions. This combination may be implemented in specific clinical settings to rule out (un)intentional gluten consumption or gluten cross-contamination, namely in children (1) with recently diagnosed with CD as they familiarize themselves with the GFD, (2) reporting symptoms with normal TGA and no errors in the dietary questionary, (3) with (persistent) elevated or very slow normalisation of TGA-levels despite no errors in the dietary questionnaire and, (4) with suspected intentional gluten intake.

REFERENCES

1. Myléus A, Reilly NR, Green PHR. Rate, Risk Factors, and Outcomes of Nonadherence in Pediatric Patients With Celiac Disease: A Systematic Review. *Clin Gastroenterol Hepatol*; 2020;18(3):562-73.
2. Itzlinger A, Branchi F, Elli L, et al. Gluten-Free Diet in Celiac Disease—Forever and for All?. *Nutrients.* 2018;10(11):1796.
3. Vriezinga SL, Farih N, van der Meulen-de Jong AE, et al. Comparison of Patients' and Doctor's Reports on Health-related Quality of Life in Celiac Disease. *J Pediatr Gastroenterol Nutr.* 2017: 64 (5): 737-41.
4. White L, Bannerman E, Gillett P. Celiac disease and the gluten-free diet: a review of the burdens; factors associated with adherence and impact on health-related quality of life, with specific focus on adolescence. *Journal of Human Nutrition and Dietetics.* 2016;29(5):593-606.
5. Wessels MMS, Te Linterlo M, Vriezinga SL, et al. Assessment of dietary compliance in celiac children using a standardized dietary interview. *Clin Nutr*. 2018; 37 (3): 1000-4.
6. Silvester JA, Comino I, Rigaux LN, et al. Exposure sources, amounts and time course of gluten ingestion and excretion in patients with celiac disease on a gluten-free diet. *Aliment Pharmacol Ther* 2020 Nov;52(9):1469-79.
7. Isaac DM, Rajani S, Yaskina M, et al. Antitissue transglutaminase normalization postdiagnosis in children with celiac disease. *J Pediatr Gastroenterol Nutr* 2017; 65:195–199.
8. Gerasimidis K., Zafeiropoulou K., Mackinder M., et al. Comparison of Clinical Methods With the Faecal Gluten Immunogenic Peptide to Assess Gluten Intake in Celiac Disease. *Journal of Pediatric Gastroenterology and Nutrition*, 2018; 67(3), 356–360.
9. Wessels MMS, Dolinsek J, Castillejo G, et al. Follow-up practices for children and adolescents with celiac disease: Results of an international survey. *European Journal of Pediatrics*. 2021; 2021 Nov 24.
10. Stefanolo JP, Tálamo M, Dodds S, et al. Real-World Gluten Exposure in Patients With Celiac Disease on Gluten-Free Diets, Determined From Gliadin Immunogenic Peptides in Urine and Fecal Samples. *Clin Gastroenterol Hepatol*. 2021;19(3):484-91.
11. Moreno M, Cebolla A, Muñoz-Suano A, et al. Detection of gluten immunogenic peptides in the urine of patients with celiac disease reveals transgressions in the gluten-free diet and incomplete mucosal healing. *Gut* 2017; 66:250–257 4.
12. Moreno M, Rodríguez-Herrera A, Sousa C, et al. Biomarkers to Monitor Gluten-Free Diet Compliance in Celiac Patients. *Nutrients.* 2017;9(1):46.
13. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr*; 2012;54(1):136-60.
14. Sansotta N, Alessio MG, Norsa L, et al. Trend of antitissue transglutaminase antibody normalization in children with celiac disease started on gluten-free diet: a comparative study between chemiluminescence and ELISA serum assays. *J Pediatr Gastroenterol Nutr*; 2020; 70:37–41.
15. Hogen Esch CE, Wolters VM, Gerritsen SA, et al. Specific celiac disease antibodies in children on a gluten-free. *Pediatrics*; 2011; 128:547–552.
16. Comino I, Fernández-Bañares F, Esteve M, et al. Fecal Gluten Peptides Reveal Limitations of Serological Tests and Food Questionnaires for Monitoring Gluten-Free Diet in Celiac Disease Patients. *American Journal of Gastroenterology.* 2016;111(10):1456-1465.
17. Werkstetter KJ, Korponay-Szabó IR, Popp A, et al. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. *Gastroenterology.* 2017;153(4):924-935.

18. Vahedi K, Mascart F, Mary J, et al. Reliability of Antitransglutaminase Antibodies as Predictors of Gluten-Free Diet Compliance in Adult Celiac Disease. *The American Journal of Gastroenterology.* 2003;98(5):1079-1087.
19. Ruiz-Carnicer Á, Garzón-Benavides M, Fombuena B, et al. Negative predictive value of the repeated absence of gluten immunogenic peptides in the urine of treated celiac patients in predicting mucosal healing: new proposals for follow-up in celiac disease. *Am J Clin Nutr.* 2020 Nov 11;112(5):1240-1251. doi: 10.1093/ajcn/nqaa188.
20. Comino I, Segura V, Ortigosa L, et al. Prospective longitudinal study: use of faecal gluten immunogenic peptides to monitor children diagnosed with celiac disease during transition to a gluten-free diet. *Aliment Pharmacol Ther*. 2019 Jun;49(12):1484-1492. doi: 10.1111/apt.15277. Epub 2019 May 10
21. Porcelli B, Ferretti F, Biviano I, et al. Testing for fecal gluten immunogenic peptides: a useful tool to evaluate compliance with gluten-free diet by celiacs. *Annals of Gastroenterology* 2020; 33, 631-637
22. Costa AF, Sugai E, de la Paz Temprano M, et al. Gluten immunogenic peptide excretion detects dietary transgressions in treated celiac disease patients. *World J Gastroenterol*. 2019; 25(11):1409-20.

7

Utilization and effectiveness of E-health technology in the follow-up of celiac disease: A systematic review

JPGN 2022 Jun1;74(6):812-818

Alice Loft Månsson
Caroline R. Meijer
Karl Mårild

ABSTRACT

Objectives: To systematically review the literature on the utilization and effectiveness of electronic-health technologies (E-health), such as smartphone applications, in managing patients with celiac disease (CD).

Methods: PubMed, Scopus, and the Cochrane Library were all searched (until February 2021). Inclusion criteria were full-text English articles reporting original data on the use of E-health technologies in the follow-up of CD patients, with no age restriction. Exclusion criteria were studies only using non-interactive websites and phone consultation as the primary E-health method. The results were summarized narratively.

Results: Using identified keywords, 926 unique studies were identified. After title and abstract screening by two independent reviewers, 26 studies were reviewed in full text. Finally, eight studies were included in this systematic review, and their quality appraised using standardized forms. Of the eight studies, six were randomized-controlled trials, one mixed-methods study, and one cross-sectional, observational study. Studies were assessed to be of "low" to "moderate" methodological quality. Studied E-health technologies included web-based interventions, smartphone applications, text messaging, and online consultations. The most consistently reported effects were related to improved quality of life (number of studies=4), knowledge on CD (n=3), and dietary adherence (n=2); notably, only one study reported reduced costs of E-health vs. standard (in-office) care.

Conclusions: While E-health has the potential to improve the management of CD, so far, the research in the field is scarce and generally of low-moderate methodological quality. Hence, the effectiveness of E-health in CD management remains uncertain, and more high-quality evidence is required before its utility is known.

INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated disease in which gluten intake causes small-intestinal inflammation and villus atrophy(1). Over the past few decades, there has been a rise in the prevalence of CD, which today affects about 1% of the population worldwide.(2) The disease is associated with various intestinal and extra-intestinal manifestations, including impaired growth and quality of life (QoL),(2) as well as increased costs on individual and societal levels (3, 4). A strict gluten-free diet (GFD) is a cumbersome but effective treatment that can alleviate symptoms and achieve mucosal healing in CD.(5) Patients with CD are recommended long-term follow-up to monitor disease remission and dietary adherence.(6) Electronic-health technologies (E-health) are defined as the use of information and communication technologies, such as software and smartphone applications, supporting health and disease management.(7) Research on E-health technologies has shown positive effects in managing a variety of chronic diseases, including asthma and type 1 diabetes.(8-10) In the care of digestive diseases,(11) including inflammatory bowel disease,(12, 13) E-health technologies have more specifically been reported to improve the patient's QoL and treatment adherence. Besides enhancing the quality of care, E-health holds the potential to reduce costs in healthcare.(14) Although CD is a major public health problem, and despite the potential benefits of E-health technologies in chronic disease management,(15, 16) the evidence of their utility and effectiveness in CD management has not yet been reported. Hence, we aimed to systematically review the literature on the utilization and effectiveness of E-health in the management of patients with CD.

METHODS

Literature search

This systematic literature review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.(17) The literature search was conducted with the support of a professional librarian using PubMed, Scopus, Cochrane Library, and Database of Abstracts of Reviews of Effectiveness. Appropriate search terms were identified from the Swedish version of Medical Subject Headings (MeSH) and the index terms of identified relevant articles. The PubMed search string is presented in Supplementary Table 1, http://links.lww.com/MPG/C720 (the same search strategy was modified to fit the requirements of the other databases).

Eligibility screening

Articles identified until February 2021 were screened against the following, predetermined, eligibility criteria. Articles of any E-health technology used in CD care were deemed eligible. However, telephone consultations (regarded as an established part of standard healthcare) and non-interactive educational websites were not considered E-health, and such articles were hence excluded.(7) Articles limited to non-original data (e.g., reviews), and full-text articles not available in English were also excluded. No further restrictions were applied. Hence, no restriction was made regarding study designs, sample characteristics (e.g., participant's age [children and adults]), outcome measures, and criteria for CD diagnosis.

The article search is depicted in Figure 1 (PRISMA flowchart). Briefly, after duplicates had been removed, articles identified in our database search were screened for eligibility in a twostep process: First, titles and abstracts were screened for eligibility by two independent reviewers (ALM and CMB). The screening was performed in Rayyan, a web application for systematic reviews,(18) where inclusion-exclusion decisions of each reviewer were blinded. Disagreements were resolved in discussion with a third reviewer (KM). Second, full-text articles were retrieved and assessed for eligibility by at least two reviewers (ALM and CMB, in case of disagreements by review of KM). Finally, the reference lists of included studies were screened for additional articles using publication titles (ALM). However, no additional article was identified through this hand searching by title (Figure 1).

Data extraction of included articles

Data from included articles were extracted using a predesigned form and synthesized narratively according to published guidance.(19) The following data were extracted from each article: Study design, sample characteristics (e.g. age, duration of CD diagnosis), number of participants, comparison group, type of E-health technology (e.g. web-based, virtual clinic, etc.), outcome measure, duration of follow-up, and main findings (Table 1). The findings were also reported by the main outcome categorizes (GFD adherence, knowledge about CD and GFD, QoL, patient satisfaction, and other outcomes).

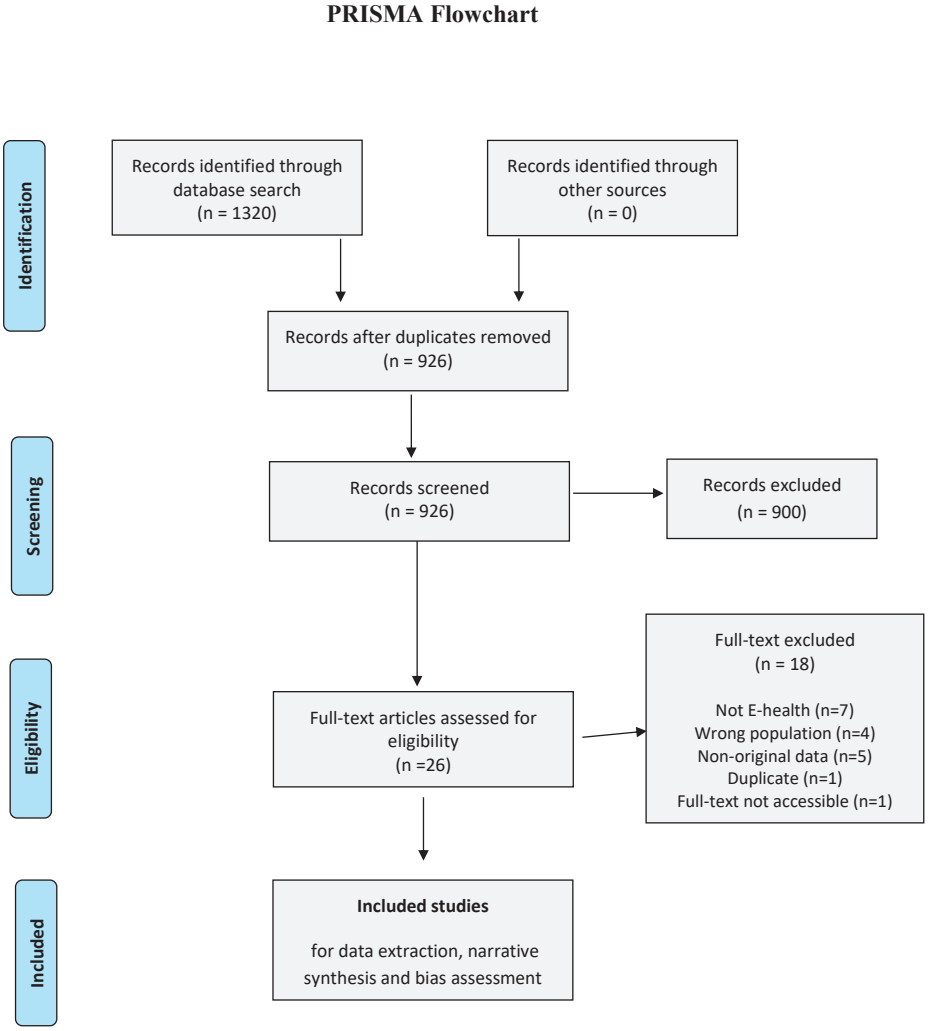


Figure 1. Flowchart of article search and eligibility screening. Literature search within PubMed, Scopus, Cochrane Library and Database of Abstracts of Reviews of Effectiveness (until February 2021). The PubMed search string is presented in Supplementary Table 1. Inclusion criteria were full-text English articles reporting original data on the use of E-health technologies in the follow-up of celiac patients, with no age restriction. Exclusion criteria were studies only using non-interactive educational websites and phone consultation as the primary E-health method.

Bias assessment of included articles

The risk of bias was according to the study design assessed by the revised Cochrane Risk-of-Bias tool for randomized-controlled trials,(20) the Mixed Methods Appraisal Tool,(21) and Joanna Briggs Institute Checklist for Analytical Cross-sectional Studies. (22) The risk of bias assessment was made in agreement with all reviewers (ALM, CMB, KM), and each article was categorized into “low-”, “moderate-” and “high-risk” of bias

Table 1. Included articles on electronic-health technologies (E-health) used in celiac disease (CD) follow-up care.

Authors, Country, (Year of publication)	Design	Participants			Type of E-health	Outcome measures	Follow-up	Main findings
		CD group	Number	Comparison				
Connan *et al.* Canada (2019)(23)	Mixed methods study	Children (mean age 14y) with CD and T1D or their caregivers	18	None	Interactive eLearning module	Satisfaction score, knowledge score.	None	eLearning module efficient for knowledge retention and information access
Dowd *et al.* Canada (2020)(26)	RCT	Adults (>18y) with CD or "gluten intolerance"	115	Wait-list control	Smartphone app, MyHealthyGut	Satisfaction, GFD adherence, QoL, self-regulatory efficacy, anxiety	One month	Improved QoL. Reduced anxiety.
Haas *et al.* USA (2017)(29)	RCT	Adolescents (ages 12-24y) with CD ≥ one year	61	Standard of care	Text Message Educational Automated Compliance Help (TEACH). 45 unique text messages	Primary: CD-specific serology); secondary: GFD adherence (CDAT), patient activation, symptoms, QoL (PROMIS global physical and mental health scales)	Three months	Significantly improved patient activation measurement score, and QoL
Lasa *et al.* Spain (2019)(28)	cross-sectional observational study	Children (ages 4-15y) and adults (23-71y) with CD ≥ one year	Not specified	Other software not containing GFD products	Software for GFD education and nutritional evaluation	GFD energy and nutrient contents	None	GFD evaluation software is a useful tool for GFD nutritional evaluation)
Meyer *et al.* Germany (2003) (24)	RCT	Patients with CD**	64	Standard of care (conventional GFD training)	Computer-based interactive training program	GFD knowledge	Three weeks	Significantly improved GFD knowledge and sustainability

Table 1. Included articles on electronic-health technologies (E-health) used in celiac disease (CD) follow-up care. (*continued*)

Authors, Country, (Year of publication)	**Design**	**Participants**			**Type of E-health**	**Outcome measures**	**Follow-up**	**Main findings**
		CD group	Number	Comparison				
Nikniaz *et al.* Iran (2021)(27)	RCT	Adults (mean age 37y) with CD ≥ six months	60	Standard of care (conventional CD/GFD training)	Persian language smartphone app	CD knowledge, GFD adherence (CDAT)	Three months	Significantly improved GFD adherence. No significant effect on CD knowledge
Sainsbury *et al.* Australia (2013)(25)	RCT	Adults (>16y) with CD	189	Wait-list control	Interactive online intervention	Primary: GFD adherence (CDAT); secondary: GFD knowledge, QoL, psychological symptoms	Three months	Significantly improved GFD adherence and knowledge, that was maintained during follow up.
Vriezinga *et al.* Netherlands (2018)(30)	RCT	Patients ≤25 years with CD ≥ one year	304	Standard of care (in-office consultations)	Online consultation	Primary: CD remission by TTG levels (Point-of-Care test/lab) Secondary: QoL, GFD adherence, patient satisfaction, cost of care	Six months	Improved CD-specific QoL, lower average costs (€93) Sign decreased satisfaction score in intervention group compared to controls (but 48% regarded the online consultation to be as good as outpatient care)

* Primary and secondary outcome measures are detailed if specified in the study.

**Age of participants not reported.

Details on the E-health technologies used in included studies are provided in the Supplementary Results. CDAT, Celiac dietary adherence test; GFD, Gluten-free diet; PROMIS, Patient reported outcome measurement and information system project; RCT, Randomized-controlled trial; TTG, anti-Tissue transglutaminase antibodies

studies. A meta-analysis was not conducted due to heterogeneity between studies. The main reasons for variations between studies were differences in the E-health used (e.g., web-based, virtual clinic, etc.) and outcome measures. Further, there were differences in populations investigated, study designs, and observation periods.

RESULTS

Using identified keywords, 926 unique studies were identified in February 2021. Based on information in the abstracts and titles, 26 studies were reviewed in full text, out of which eight studies(23-30) eventually formed the basis of this systematic review (Figure 1, PRISMA Flowchart). The studies included six RCTs,(24-27, 29, 30) one mixed-methods study,(23) and one cross-sectional observational study (Table 1)(28). Included studies were assessed to be of "low" to "moderate" methodological quality (Supplementary Tables 2 and 3, http://links.lww.com/MPG/C721). Total 811 participants were included in the articles (one study did not report its sample size(28)); four of the eight studies were restricted to adults,(24-27) two included both adults and children,(23) (28) while two studies included only children and adolescents(29, 30). Of the eight included studies, three examined web-based interventions(23-25), three smartphone applications,(26-28) one a telemedicine (text messaging) intervention,(29) and one study examined the use of online consultations (i.e., a virtual clinic)(30). Details on the E-health technologies used in included studies are provided in the Supplementary Results, http://links.lww.com/MPG/C719.

Gluten-free dietary adherence

Five studies(25-27, 29, 30) investigated the effects of E-health interventions on GFD adherence. Of these, two studies, one examining a smartphone application,(27) and one an interactive online training program,(25) reported significantly improved adherence rate in the intervention group compared to baseline measurements and post-intervention controls. Both these studies included adult celiac patient who may had been relatively newly diagnosed (minimum 3-6 months since diagnosis).(27) (25) In contrast, Dowd et al.,(26) examining the effect of a CD self-management application, surprisingly reported that both the intervention group (adult patients with unspecified disease duration) and controls had significantly worsened GFD adherence rate compared to baseline. Neither of the RCTs performed by Haas et al. and Vriezinga et al. found an effect from their interventions on GFD adherence.(29, 30) Both these RCTs were conducted on children or young adults (age <25 years) with a minimum disease duration of one year.

Knowledge about celiac disease and gluten-free diet

Four studies examined the effect of E-health on CD and GFD knowledge.(23-25, 27) All except one study(27) reported significant improvements in CD and/or GFD knowledge of the intervention groups. Connan et al.,(23) including children with a dual diagnosis of CD and type 1 diabetes, saw a small but statistically significant improvement on a CD and GFD knowledge test after an E-learning intervention compared to baseline (p-value=0.001); the Elearning intervention seemed particularly effective in knowledge retention and to provide comprehensive and easily accessible information on GFD. Also, Meyer et al.(24) reported significantly improved knowledge about CD and GFD in adults using a computer-based interactive training program compared to a conventional training program (although both study groups improved their knowledge scores). In the study by Sainsbury et al.,(25) GFD knowledge scores were significantly enhanced in adults using an interactive training program compared to controls. The study by Nikniaz et al. saw no significant effect of their smartphone application on CD and GFD knowledge in adults compared to controls receiving conventional training.(27)

Quality of life

Four studies examined improvements in QoL in CD through E-health technology, one study using a text-message intervention,(29) one a smartphone application,(26) one online consultation,(30) and one study using an online, interactive GFD training program.(25) Two of the studies were restricted to adults,(26) (25) and two on children and adolescents (29) (30). Despite also variations in used E-health technologies as well as QoL instruments used, all studies reported significant positive effects on the QoL of celiac patients; however, in the study of Sainsbury et al.(25) (GFD training program), the improvement was limited to specific aspects of QoL (physical and psychological domains) and not the overall QoL, including for instance also aspects related to social relationships and independence.

Patient satisfaction

Patient satisfaction was measured by questionnaires or semi-structured interviews in three studies using the following E-health technologies: smartphone application,(26), an interactive online training program,(23) and online consultations (30). Most,(23, 26) but not all,(30) studies reported participant satisfaction from using respectively examined E-health technology. Connan et al.(23) reported overall high satisfaction scores with their online GFD education toll used by children (and their caregivers). While Dowd et al.(26) reported that adults with prevalent CD generally found the MyHealthyGut application satisfactory, the participants also said they were unlikely to purchase it or continue using this application after the study. This reluctance was suggested to be related to the application being tailored to more newly diagnosed CD. In contrast, chil-

dren and young adults (aged 2.6-24.1 years) receiving online follow-up care in the study by Vriezinga et al.(30) were significantly less satisfied compared to controls receiving standard (in-office) consultations (p-value=0.001). Compared to controls, participants experienced virtual consultations more impersonal, but found their location and timing more convenient (all p-values<0.02); one third of the intervention group experienced technical problems resulting in lower satisfaction. On the other hand, 48% of the intervention group considered online consultation as equally good as in-office follow-up visits, and 58% wished to continue with online consultations at the end of the study.

Other outcomes

Only one study compared the cost of E-health technology to standard of care. Vriezinga et al.(30) found online follow-up consultations to be, on average, €93 less costly than in-office follow-up (total costs, €143 vs. €236, p-value <0.001). The impacts of E-health on mental health in CD were investigated in two studies.(25, 26) Dowd et al.(26) saw a significant decrease in anxiety measurement one month after the intervention compared to baseline. On the other hand, Sainsbury et al.(25) reported no significant effects from their CD smartphone application on measures of depression and anxiety (all p-values >0.05).

Finally, Haas et al.(29) reported a significant improvement in celiac patient activation (i.e., the ability to self-manage the disease(31)) by the use of a text-message intervention tailored for the disease.

DISCUSSION

This first systematic review on the use of E-health specifically for CD care identified eight studies:(23-30) six RCTs,(24-27, 29, 30) one mixed-methods,(23) and one observational study.(28) Most included studies concluded patient satisfaction with E-health and that its use may be effective in specific aspects of CD care; improved QoL,(25, 26, 29, 30) adherence rate,(25, 27) and knowledge on CD and GFD were among the most consistent findings of this review.(23-25) We found examined E-health interventions to improve the QoL of both pediatric and adult celiac patients.(25, 26, 29, 30) This positive effect on QoL aligns with the results from E-health interventions for other chronic diseases,(11, 13) and may be related to E-health's potential to strengthen the opportunities for patient activation and health education.(32) Speculatively, the better QoL in celiac patients may also be related to an improved GFD adherence rate from studied E-health interventions.(25) Despite inconsistent methodology and outcome measures, two of the included studies reported significantly improved GFD adherence.(25, 27) This finding, though only from

two studies, is encouraging given that strict GFD is a cumbersome treatment, where the prolonged intake of only trace amounts of gluten can cause symptoms,(33) nutritional deficiencies and prevent mucosal healing in CD.(5, 34) Unexpectedly, Dowd et al.(26) found significantly higher non-adherence rates in both intervention and control groups; the reason for this worsened adherence rate is unknown but may be related to the fact that participants at baseline already had an "excellent" to "very good" adherence level. Generally, E-health interventions seem effective in improving nutritional behaviors (e.g., a decreased fat intake and increased intake of fruits and vegetables)(35) and nutritional-related outcomes (e.g., obesity).(36) Disease knowledge is a prerequisite for a patient to manage a chronic disease successfully.(37) The beneficial effects of E-health on CD and GFD knowledge were among the most consistent findings of this systematic review (reported by three out of four studies with that outcome).(23-25) The study by Nikniaz et al.,(27) which did not see an improved CD knowledge, differs from the other studies in using a smartphone application rather than an interactive eLearning/training module. (23-25) However, it is unknown if that difference also explains the difference in results. The use of E-health has for other autoimmune conditions (e.g., type 1 diabetes) been shown to improve illness-related knowledge.(38)

Challenges of implementing E-health into CD care

From our results,(26) and others,(13) it is conceivable that various E-health technologies may better fit the needs of specific groups of CD patients, e.g., defined by disease duration, age and level of disease control. If such associations could be established, it would be possible to tailor virtual CD care to patients' characteristics and specific needs. For instance, while data are limited, we noted that E-health interventions on GFD adherence have so far been more successful in studies of relatively newly diagnosed adult patients rather than studies of children with >1-year disease duration.(27) (25, 29, 30) Barriers to E-health implementation include technology illiteracy and poor internet acceptability. Hence, the adoption and perceived usefulness of E-health for CD could also have geographical differences. A low income and education level have been associated with reduced internet access and use of E-health technology.(39, 40) This has led to concerns that the increased use of digital care might exacerbate socioeconomic gaps in care access. Further, E-health technology has been found to be less accessible to individuals living in rural areas (41) limiting the potential benefits of such interventions on lowering costs and time of travel to care sites. Finally, compared to younger adults, limited internet access and E-health literacy are more common among older people. (40) This age-based disparity is noteworthy given that CD has become increasingly more recognized as a disease in the elderly.(42) However, even younger celiac patients may not necessarily equally appreciate virtual clinics as stand-alone tools for follow-up, compared to in-office care.(30)

Cost reduction is a frequent argument for the implementation of E-health. Indeed, a 2014 umbrella review of E-health in somatic diseases (not including CD) indicated cost-effectiveness,(14) however, this was not a consistent finding and has been contradicted by others.(43) In Colorado, USA, having online access to clinicians was associated with increased use and cost of clinical services compared to those without online access.(44) In the so far only cost-benefit analysis of E-health in CD management, Vriezinga et al.(30) showed a small but significantly decreased cost of a virtual clinic compared to standard outpatient-led care (average cost reduction €93). However, the analysis did not include costs for developing and maintaining the information and communication system required for online consultation, which has constituted a large part of the total cost in other studies.(45) Future cost-benefit analyses of E-health vs. standard follow-up of CD should also include long-term costs, which largely depend on the successful prevention of comorbidities and complications to CD.(3) It is also unknown if E-health may reduce work loss in CD.(4)

Strengths and limitations: Strengths of this study include a thorough literature search in multiple databases using a comprehensive search string. The latter is essential as there are no universal MeSH term indexing E-health studies. A restricted search strategy could hence increase the risk of not identifying all available data. Further, we applied minimal restrictions to our eligibility screening to include all relevant studies in the field, irrespective of patient ages, E-health technology studied, etc. Our use of standardized quality assessment forms and independent reviewers for literature screening and data extraction are additional strengths. This study was mainly limited by the low number of E-health studies on CD care published so far. This scarcity of data, of low-moderate methodological quality, prevented firm conclusions on the effectiveness and utility of E-health in CD care. The lack of data, and their heterogeneity in terms of examined E-health technology and outcome measures, also impeded a meta-analysis of results. The novel coronavirus 2019 pandemic has presented the healthcare system with unprecedented challenges that have necessitated a rapid adaptation to remote care delivery.(46, 47) This transformation of healthcare delivery, including the rise of virtual CD care, should spur more research in this field to support the successful implementation of this technology.

Future research directions

We did not identify any low-risk of bias study for this review (Supplementary Tables 2, http://links.lww.com/MPG/C721 -3, http://links.lww.com/MPG/C722). This highlights a need for higher-quality research on E-health in CD care. Common methodological shortcomings of included studies that should be tackled in future works include high attrition rates and improper or poorly described randomization or blinding procedures.

Opportunities for future research also include a wider use of quantitative disease control measures, such as CD-specific serologies or gluten-immunogenic peptides.(48) Finally, there is a paucity of research analysing the contents and quality of health-promoting applications.(49, 50) Hence, future research should try to assess commonalities ("success factors") in high-effective E-health interventions in CD care.

Conclusions

Although individual E-health studies have shown improvements for specific aspects of CD care, such as QoL and CD knowledge, there are so far insufficient data and a heterogeneity in study methods and targeted outcomes to determine the effectiveness and utility of E-health in CD care. This knowledge gap, combined with increasing demands on healthcare services to provide remote care, should be an incentive for more research.

REFERENCES

1. Lebwohl B, Sanders DS, Green PHR Celiac disease. Lancet 2018;391(10115):70-81.
2. Leonard MM, Sapone A, Catassi C, et al. Celiac Disease and Nonceliac Gluten Sensitivity: A Review. JAMA 2017;318(7):647-56.0
3. Marild K, Soderling J, Bozorg SR, et al. Costs and Use of Health Care in Patients With Celiac Disease: A Population-Based Longitudinal Study. Am J Gastroenterol 2020;115(8):1253-63.
4. Bozorg SR, Soderling J, Everhov AH, et al. Work Loss in Patients With Celiac Disease: A Population-based Longitudinal Study. Clin Gastroenterol Hepatol 2021.
5. Myleus A, Reilly NR, Green PHR Rate, Risk Factors, and Outcomes of Nonadherence in Pediatric Patients With Celiac Disease: A Systematic Review. Clin Gastroenterol Hepatol 2020;18(3):562-73.
6. Husby S, Murray JA, Katzka DA AGA Clinical Practice Update on Diagnosis and Monitoring of Celiac Disease-Changing Utility of Serology and Histologic Measures: Expert Review. Gastroenterology 2019;156(4):885-89.
7. WHO. WHO guideline Recommendations on Digital Interventions for Health System Strengthening. Geneva; 2019.
8. Jeminiwa R, Hohmann L, Qian J, et al. Impact of E-health on medication adherence among patients with asthma: A systematic review and meta-analysis. Respir Med 2019;149(59-68).
9. Duke DC, Barry S, Wagner DV, et al. Distal technologies and type 1 diabetes management. Lancet Diabetes Endocrinol 2018;6(2):143-56.
10. Deacon AJ, Edirippulige S Using mobile technology to motivate adolescents with type 1 diabetes mellitus: A systematic review of recent literature. J Telemed Telecare 2015;21(8):431-8.
11. Helsel BC, Williams JE, Lawson K, et al. Telemedicine and Mobile Health Technology Are Effective in the Management of Digestive Diseases: A Systematic Review. Dig Dis Sci 2018;63(6):1392-408.
12. Ankersen DV, Carlsen K, Marker D, et al. Using E-health strategies in delivering dietary and other therapies in patients with irritable bowel syndrome and inflammatory bowel disease. J Gastroenterol Hepatol 2017;32 Suppl 1(27-31.
13. Jackson BD, Gray K, Knowles SR, et al. E-health Technologies in Inflammatory Bowel Disease: A Systematic Review. J Crohns Colitis 2016;10(9):1103-21.
14. Elbert NJ, van Os-Medendorp H, van Renselaar W, et al. Effectiveness and costeffectiveness of E-health interventions in somatic diseases: a systematic review of systematic reviews and meta-analyses. J Med Internet Res 2014;16(4):e110.
15. Penedo FJ, Oswald LB, Kronenfeld JP, et al. The increasing value of E-health in the delivery of patient-centred cancer care. Lancet Oncol 2020;21(5):e240-e51.
16. Schneider RB, Biglan KM The promise of telemedicine for chronic neurological disorders: the example of Parkinson's disease. Lancet Neurol 2017;16(7):541-51.
17. Yepes-Nunez JJ, Urrutia G, Romero-Garcia M, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Rev Esp Cardiol (Engl Ed) 2021;74(9):790-99.
18. Ouzzani M, Hammady H, Fedorowicz Z, et al. Rayyan-a web and mobile app for systematic reviews. Syst Rev 2016;5(1):210.
19. Popay J, Roberts H, Sowden A, et al. Guidance on the Conduct of Narrative Synthesis in Systematic Reviews: Final Report. Swindon: ESRC Methods Programme 2006.
20. Cumpston M, Li T, Page MJ, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. Cochrane Database Syst Rev 2019;10(ED000142.

21. Hong QN, Gonzalez-Reyes A, Pluye P Improving the usefulness of a tool for appraising the quality of qualitative, quantitative and mixed methods studies, the Mixed Methods Appraisal Tool (MMAT). J Eval Clin Pract 2018;24(3):459-67.
22. Moola S, Munn Z, Tufanaru C, et al. Chapter 7: Systematic reviews of etiology and risk In: Aromataris E and M. Z eds. Joanna Briggs Institute Reviewer's Manual. The Joanna Briggs Institute; 2017.
23. Connan V, Marcon MA, Mahmud FH, et al. Online education for gluten-free diet teaching: Development and usability testing of an e-learning module for children with concurrent celiac disease and type 1 diabetes. Pediatr Diabetes 2019;20(3):293-303.
24. Meyer KG, Fasshauer M, Nebel IT, et al. Comparative analysis of conventional training and a computer-based interactive training program for celiac disease patients. Patient Educ Couns 2004;54(3):353-60.
25. Sainsbury K, Mullan B, Sharpe L A randomized controlled trial of an online intervention to improve gluten-free diet adherence in celiac disease. Am J Gastroenterol 2013;108(5):811-7.
26. Dowd AJ, Warbeck CB, Tang KT, et al. MyHealthyGut: Findings from a pilot randomized controlled trial on adherence to a gluten-free diet and quality of life among adults with celiac disease or gluten intolerance. Digit Health 2020;6(2055207620903627.
27. Nikniaz Z, Shirmohammadi M, Akbari Namvar Z Development and effectiveness assessment of a Persian-language smartphone application for celiac patients: A randomized controlled clinical trial. Patient Educ Couns 2021;104(2):337-42.
28. Lasa A, Larretxi I, Simon E, et al. New Software for Gluten-Free Diet Evaluation and Nutritional Education. Nutrients 2019;11(10).
29. Haas K, Martin A, Park KT Text Message Intervention (TEACH) Improves Quality of Life and Patient Activation in Celiac Disease: A Randomized Clinical Trial. J Pediatr 2017;185(62-67 e2.
30. Vriezinga S, Borghorst A, van den Akker-van Marle E, et al. E-healthcare for Celiac Disease-A Multicenter Randomized Controlled Trial. J Pediatr 2018;195(154-60 e7.
31. Hibbard JH, Stockard J, Mahoney ER, et al. Development of the Patient Activation Measure (PAM): conceptualizing and measuring activation in patients and consumers. Health Serv Res 2004;39(4 Pt 1):1005-26.
32. Ossebaard HC, Van Gemert-Pijnen L E-health and quality in health care: implementation time. Int J Qual Health Care 2016;28(3):415-9.
33. Catassi C, Fabiani E, Iacono G, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr 2007;85(1):160-6.
34. Rubio-Tapia A, Rahim MW, See JA, et al. Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. Am J Gastroenterol 2010;105(6):1412-20.
35. Olson CM Behavioral Nutrition Interventions Using e- and m-Health Communication Technologies: A Narrative Review. Annu Rev Nutr 2016;36(647-64).
36. Villinger K, Wahl DR, Boeing H, et al. The effectiveness of app-based mobile interventions on nutrition behaviours and nutrition-related health outcomes: A systematic review and meta-analysis. Obes Rev 2019;20(10):1465-84.
37. Gazmararian JA, Williams MV, Peel J, et al. Health literacy and knowledge of chronic disease. Patient Educ Couns 2003;51(3):267-75.
38. Bertuzzi F, Stefani I, Rivolta B, et al. Teleconsultation in type 1 diabetes mellitus (TELEDIABE). Acta Diabetol 2018;55(2):185-92.
39. Latulippe K, Hamel C, Giroux D Social Health Inequalities and E-health: A Literature Review With Qualitative Synthesis of Theoretical and Empirical Studies. J Med Internet Res 2017;19(4):e136.

40. Chesser A, Burke A, Reyes J, et al. Navigating the digital divide: A systematic review of E-health literacy in underserved populations in the United States. Inform Health Soc Care 2016;41(1):1-19.
41. Currie M, Philip LJ, Roberts A Attitudes towards the use and acceptance of E-health technologies: a case study of older adults living with chronic pain and implications for rural healthcare. BMC Health Serv Res 2015;15(162.
42. Rashtak S, Murray JA Celiac disease in the elderly. Gastroenterol Clin North Am 2009;38(3):433-46.
43. Sanyal C, Stolee P, Juzwishin D, et al. Economic evaluations of E-health technologies: A systematic review. PLoS One 2018;13(6):e0198112.
44. Palen TE, Ross C, Powers JD, et al. Association of online patient access to clinicians and medical records with use of clinical services. JAMA 2012;308(19):2012-9.
45. Grey M, Liberti L, Whittemore R Costs of Development and Maintenance of an Internet Program for Teens with Type 1 Diabetes. Health Technol (Berl) 2015;5(2):127-33.
46. Wosik J, Fudim M, Cameron B, et al. TelE-health transformation: COVID-19 and the rise of virtual care. J Am Med Inform Assoc 2020;27(6):957-62.
47. Dobrusin A, Hawa F, Gladshteyn M, et al. Gastroenterologists and Patients Report High Satisfaction Rates With TelE-health Services During the Novel Coronavirus 2019 Pandemic. Clin Gastroenterol Hepatol 2020;18(11):2393-97 e2.
48. Stefanolo JP, Talamo M, Dodds S, et al. Real-World Gluten Exposure in Patients With Celiac Disease on Gluten-Free Diets, Determined From Gliadin Immunogenic Peptides in Urine and Fecal Samples. Clin Gastroenterol Hepatol 2021;19(3):484-91 e1.
49. Armstrong S Which app should I use? BMJ 2015;351(h4597.
50. Bardus M, van Beurden SB, Smith JR, et al. A review and content analysis of engagement, functionality, aesthetics, information quality, and change techniques in the most popular commercial apps for weight management. Int J Behav Nutr Phys Act 2016;13(35)

8

General discussion and conclusions

Celiac disease (CD) is an immune-mediated disorder, in which the HLA immunogenetic background (DQ2 and DQ8 heterodimers) and environmental trigger (gluten) are well established. Both factors are necessary- but not sufficient- to develop CD.

CD is a common disease with a broad spectrum of intestinal and extraintestinal symptoms and potential complications like osteoporosis, autoimmunity and rare but severe malignancies. The prevalence of CD is increasing, which has been mainly attributed to the greater availability of sensitive and specific screening tests, the growing awareness of CD among health-professionals and identification of those at risk of CD which have led to a significant raise in diagnoses worldwide (1).

Despite the fact that knowledge about the pathophysiology, diagnosis, treatment and possible therapeutic options is gradually increasing, it remains unclear who develops CD and who does not. Timely diagnosis and adequate treatment and follow-up are important questions at this time and reason for the studies included in this thesis.

Since 2012 guidelines of the European society of Gastroenterology, Hepatology and Nutrition (ESPGHAN) allow for diagnosis of CD without performing small bowel biopsies in children with symptoms and levels of antibodies against tissue transglutaminase (TGA)≥ 10 x upper limit of normal (ULN), confirmed by detection of anti-endomysial antibodies (EMA) and positivity for HLA-DQ2/DQ8 (2). Prospective validation study of this approach showed positive prediction values ranged from 99.63 (95% CI, 98.67-99.96) to 100.00 (95% CI, 99.23-100.00) (3). In 2020, the Evidence based guidelines for the diagnosis of CD have been updated and published (4). In **chapter 2** of this thesis, our national prospective data show that in the Netherlands, the year after the publication of the 'non-biopsy' approach, the diagnosis was correctly established according to it in more than 75% of the children. In order to improve this, it is important that the general doctors and pediatricians who play an essential role in suspecting the diagnosis and ordering the initial serological tests, should be taken into account the recommendations made by the ESPGHAN guideline that the diagnosis always be established by a pediatric-gastroenterologists or pediatrician with sufficient experience and knowledge of CD to avoid both overdiagnosis and underdiagnosis and their consequences. In addition, due to the continuous changing clinical presentation as reported in our study, the age at time of diagnosis is significantly increasing, which makes it difficult to diagnose all children timely (5). Nevertheless, a rising incidence of childhood CD has been reported in many countries, including in the Netherlands likely caused by a combination of several factors, as the growing awareness of CD among healthcare professionals and increased screening of high-risk groups and the availability of reliable CD antibody tests (5, 6). However, also a true rise in the incidence of CD is also being considered (7), since similar increase

has been reported in other autoimmune diseases and allergic conditions in children, such as type 1 diabetes mellitus, asthma and allergic rhinitis (8-11). Understanding how tolerance to gluten is lost in CD is a fundamental question that needs more study, currently environmental factors have been linked to the rise in incidence of the disease, including viral infections during childhood or changes in gut microbiota (composition or metabolite production) (12). In **chapter 3** a review on the current knowledge of the preventive strategies of CD is presented. Advances in the pathophysiology of CD could also enable primary preventive strategies in individuals genetically predisposed to the disease, but till now, primary prevention of CD is not (yet) possible. Early infant feeding practices have been prospectively studied in this respect and it has been shown that neither the timing of gluten introduction nor the duration or maintenance of breastfeeding influence the risk of CD (13-17) Recent studies from birth cohorts of children from CD families suggest that the quantity of gluten consumed early in life, may be a (preventable) risk factor for CD development (18-20), but before 'prevention' recommendations on this aspect might be given, this topic should be studied in randomized controlled intervention trials. At this moment the definite microbial signature and the exact role of dysbiosis in CD pathogenesis is not recognized, but an association between alterations in the gut microbiota and the development of CD has been demonstrated. Results of the CDGEMM study (Celiac Disease Genomic, Environmental, Microbiome, and Metabolomic) are expected and could help to understand the role that the gut microbiome show in the early steps involved in the pathogenesis of CD (21). Knowledge of the role of intestinal bacteria in the development of CD opens new possibilities for its treatment through probiotic administration, even though further studies are needed to better clarify whether probiotics can help treat or prevent the disease and to define which probiotics to use, at what dose and for how long (22-26). As long as primary prevention of CD is not possible, diagnosing the disease in its earliest stage – secondary prevention – seems the best option. Despite the increasing numbers of diagnosed CD, a substantial number of people with CD remain undiagnosed (11), the possibility and feasibility of screening strategies to identify undetected CD patients should be explored. Major questions have emerged about who to test for CD and when. Early diagnosis may be achieved both by case finding or by mass screening, albeit both methods are still controversial because of their ethical implications (27-29). In **chapter 4** of this thesis our national project on early diagnosis of CD, GLUTENSCREEN, in children from the general population who have CD-related symptoms is presented. This concerns the first case-finding project in the Netherlands on early detection of CD, to show that it is feasible, efficient, cost-effective and well accepted by the population. In GLUTENSCREEN the parents of all the children 1-4 years old who visit the Preventive Youth Health Care Centres (YHCCs) in the region of Kennemerland for a regular consultation were asked for CD-related symptoms from a standardised list. If one or more symptoms were present, a point of care test (POCT)

for TGA was performed. If the POCT was positive, the child was referred to the Leiden University Medical Centre for diagnosis according to the guideline. The results of GLUTENSCREEN are beyond expectations: CD was confirmed in 1.8% of the tested children, more than expected on the basis of the current literature, which is approximately 1% (30). In addition, this case-finding method for early CD-diagnosis is well-accepted by the parents of young children in the Netherlands. Also, the majority of the healthcare professionals support this case-finding at the (YHCCs) (31). However, it is well known that the predictive value of symptoms to identify CD is limited, since symptoms associated with CD are as prevalent in individuals with and without the disease (32). In the database of GLUTENSCREEN, we will first examine whether some symptoms better distinguish the presence of CD or what the optimal set of symptoms is as an indication for (early) initiation of CD testing. The primary limitation of case-finding for early detection of CD, even when well implemented, is that subclinical cases will be missed (33). An alternative is a general screening program to identify all CD cases, but this was opposed in 2017 by the Medical Ethical Committee Leiden-Den Haag-Delft (METC-LDD) and The Dutch Central Committee on Research Involving Human Subjects (CCMO). On the contrary, a CD-mass-screening programme was reported as acceptable by 70% of the parents from the Dutch general population (31). Since the development of CD requires genetic susceptibility (HLA-DQ2 and/or DQ8), adding the HLA-genotype to the case finding strategy for CD could optimize the targeted population to develop CD. Currently, HLA typing is not part of GLUTENSCREEN because the existing technique requiring DNA extraction has significant drawbacks in settings without the availability of a laboratory, such as the consultation offices. However, this approach will also not solve the problem of the asymptomatic children.

But, the asymptomatic children remain undiagnosed using this strategy and will only be diagnosed with CD by mass screening. Results from the 'Generation R'-study among 6-year-old children from the general population showed in 1.3% undiagnosed CD (57/4442 screened children was positive for TG2A) and was associated with important health problems, such as a reduced bone mineral density and a delayed growth in weight (33). In addition, children of women with undiagnosed and thus untreated coeliac disease had a reduced fetal growth and a lower birth weight (34). Together with literature that mass screening for CD is cost-effective, it will be time to re-open the discussion about mass screening for CD (35-37). After many years a possible mass screening for CD is still a matter of debate. The controversy over whether the general population would accept mass screening seems to be answered by the preliminary results of GLUTENSCREEN that the majority of the general population (approximately 70%) find mass-screening for CD acceptable. Also, the results from PreventCD, a prospective, European, dietary-intervention study among infants from families with high risk of CD,

have provided valuable information about the natural course of CD. Both in children from CD families and in the general population, the development of CD is very high in the first four years of life. With the gaining of these new insights, it seems time to re-open the discussion 'is it time for mass screening' since all the ten criteria for mass-screening made by Wilson and Jungner, have finally been answered (38). Expanding CD testing in asymptomatic children will provide insight into the actual prevalence of CD and allow timely diagnosis for all children with CD. It will be a good step in the right direction for preventive care in the Netherlands.

Early CD diagnosis through target serological screening of high-risk groups, such as first-degree relatives (FDR) of CD patients and patients with autoimmune diseases, is already recommended both by the Dutch and most international guidelines (2, 39, 40). Nevertheless, little information is available about the improvement of symptoms after early diagnosis and treatment in these children. About half of these children have complaints at the time of diagnosis. In our multicenter PreventCD study children have been prospectively assessed for the development of CD by using a standardized questionnaire on their health status (reported by the parents) and CD antibodies, these data have been analysed to prospectively assess whether children from coeliac families benefit from screening, early diagnosis and treatment. These data show that symptomatic children from CD families do benefit from early detection, diagnosis and treatment. Most of the symptoms significantly improved after treatment with a GFD (manuscript in preparation).

That children from CD families have a higher risk of developing the disease, is generally known, but **in chapter 5**, our prospectively obtained data of the PREVENTCD cohort shows a significantly higher risk for the disease during the first years of life than previously assumed (1, 41). Until recently, the lifetime risk of CD for FDR of CD patients was considered to be 5%-10%. Our data show that at the age of eight years, the probability of CD is as high as 17%, stressing even more, the importance of early screening and diagnosis. However, evidence on the frequency of and at what age to perform screening, is lacking. Based on the natural course of CD, our data show that different factors influence the risk. CD develops very young age (mean age 4.3 years), significantly more often in girls ($p=0.005$) and in HLA-DQ2 homozygous individuals ($p<0.001$). Based on these factors, and the current age, prediction models for CD development were created for individualized screening advice in children with affected FDR as presented in **chapter 5**. From the findings of PREVENTCD and GLUTENSCREEN, we know now that the natural history of CD includes a very early development in life. However, whether the prediction models and resulting screening advice are also applicable in children from the general population, should be separately evaluated.

A timely diagnosis of this chronic disease is beneficial in (a)symptomatic children but to monitor the results of its treatment after diagnosis is, however, equally important. The only treatment for CD is a life-long strict gluten-free diet (GFD), which is difficult to maintain because of gluten being present in most processed foods, and dietary restrictions affect Quality of Life (QoL). In addition, gluten-free food is not widely available, it is more expensive, with lower palatability, resulting in low compliance. Many trials are underway to explore non-dietary treatment as possible options for tertiary prevention (42).

Good adherence to the GFD reduces the complications of CD and may be considered as a tertiary preventive measurement (43). Determination of TGA, which usually disappear approximately 12 months after starting a GFD, is mostly performed during the follow up and are widely used as a biomarker for mucosal healing in CD children, but the results do not correlate well with diet compliance (44). Despite the absence of a gold standard to assess dietary compliance, a dietary evaluation by a trained dietitian is considered the best method, but this is time-consuming and requires expert personnel which is not always available. Short dietary questionnaires and TGA determinations in serum fail to detect dietary transgressions in children and adolescents with CD, showing poor sensitivity to identify all patients who consume gluten (45). To assess the dietary compliance in children and adolescents with CD, a dietary questionnaire has been developed and validated (46). Other methods, as measurement of gliadin immunogenic peptides (GIP) in urine and/or in faeces have emerged as more sensitive tools to detect gluten ingestion (47), but they are not yet used in the standard clinical care. In **chapter 6,** the results of our clinical study report, that the combination of the dietary questionnaire and urinary GIP test is the most effective method in detecting (un)intentional gluten transgressions. However, both test as well as the TGA determination, have their limitations to monitor dietary compliance. Because GIP test detects gluten which is ingested only a few days prior to testing, gluten consumption before this time may remain undetected. As presented in our study, GIP determination might be helpful in specific clinical settings to rule out (un)intentional gluten intake, for example in children 1. with recently diagnosed with CD as they familiarize themselves with the GFD, 2. reporting symptoms with normal tTGA and no errors in the dietary questionary, 3. with (persistent) elevated or very slow normalisation of tTGA-levels despite no errors in the dietary questionnaire and, 4. with suspected intentional gluten intake. In contrast to the currently used biomarkers for screening and diagnosis, there is a need for a clinically useable biomarker which can assist in the monitoring of the disease over a longer period time.

Traditional medical care for CD patients consists of regular physician visits. The limited time allotted for outpatient follow-up also typically restricts comprehensive assessment

of a patient's health-related quality of life (HRQoL) and dietary adherence (4). Therefore, other possibilities outside the outpatient clinic should be considered. Self-management has shown beneficial effects on the healthcare of other chronic diseases (48). E-Health can play an important role in supporting patients in their self-management, as internet and technology can reach users easily and rapidly, with a wide range of contents and attractive formats. E-health is defined as healthcare services and information delivered or enhanced electronically via the internet and related technologies. In **chapter 7** the results are presented of a systematic review on the utilization and effectives of electronic-health technologies in the management of CD patients. The majority of the patient are satisfied with E-Health and the use may be effective in specific aspects of CD care; improved QoL, adherence rate, and knowledge on CD and GFD.

During the follow-up visits of children and adolescents with CD it is necessary to assess symptoms, nutritional status and growth, QoL and to prevent complications. Other general goals of coeliac follow-up are to ensure disease education and social support and to motivate the child and its family, reinforcing at each visit, the importance of dietary compliance. Currently, the follow-up of CD children is not standardized and based largely on expert opinions, resulting in substantial differences in follow up between countries and even regionally within countries applying the same health care system (49-50). So, there is a need for structured evidence based follow up guideline for CD. In the meanwhile, an international collaboration of experts in the field of CD has produced the ESPGHAN position paper for the management of CD. Based on available literature, recommendations have been formulated for a more structured follow-up of children with CD (51). Let's hope that these recommendations will be followed as quickly and efficiently as the ESPGHAN guideline for diagnosis of CD in children and adolescents.

FUTURE PERSPECTIVES

For now, as primary prevention of CD is a highly attractive, but as yet unrealized goal, the focus must be on driving expeditious diagnosis and treatment in (a)symptomatic children and adolescents. The preliminary results of GLUTENSCREEN show that case finding detects a significant part of the otherwise undetected children and shortens the time to diagnosis and provides us information about the cost-effectiveness and acceptability of early diagnosis in symptomatic children with CD. Based on these positive results, the YHCC's in the region of Kennemerland have decided to implement the early detection of CD in their regular care. Efforts are being made to expand the case finding approach to all other YHCCs in the Netherlands. To optimize the targeted population, funding has been requested to develop a novel test to perform HLA typing in dried blood

spots (as done at the neonatal heel-prick) obtained from an extra droplet of blood from the finger prick performed for the POCT for TGA at the YHCC. This approach will make the successful case finding more effective and reduce the burden in the HLA-DQ2/8 negative children.

In the meantime, we continue to explore opportunities for primary prevention.

REFERENCES

1. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al (2018) Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Volume 16, Issue 6, pp 823–836.
2. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012;54(1):136–60
3. Werkstetter KJ, Korponay-Szabó IR, Popp A, et al. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology. 2017;153(4):924-935.
4. Husby S, Koletzko S, Korponay-Szabó IR et al. European Society Pediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 70(1):141-156.
5. Meijer CR, Schweizer JJ, Peeters A, Putter H, Mearin ML. Efficient implementation of the 'non-biopsy approach' for the diagnosis of childhood celiac disease in the Netherlands: a national prospective evaluation 2010–2013. Eur J Pediatr. 2021; 180(8): 2485–2492.
6. Riznik P, De Leo L, Dolinsek J, Judit Gyimesi J, Martina Klemenak M, Berthold Koletzko B, et al. Clinical Presentation in Children With Coeliac Disease in Central Europe. J Pediatr Gastroenterol Nutr. 2021 Apr 1;72(4):546-551.
7. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, Absah I. Increasing incidence and altered presentation in a population-based study of pediatric celiac disease in North America. J Pediatr Gastroenterol Nutr 2017; 65:432–437
8. Lipman TH, et al. Increasing incidence of type 1 diabetes in youth: twenty years of the Philadelphia Pediatric Diabetes Registry. Diabetes care. 2013; 36(6):1597–603.
9. Lindfors, K. et al. Metagenomics of the faecal virome indicate a cumulative effect of enterovirus and gluten amount on the risk of coeliac disease autoimmunity in genetically at risk children: the TEDDY study. Gut 69, 1416–1422 (2020).
10. Loh W, Tang MLK. The Epidemiology of Food Allergy in the Global Context. Int. J. Environ. Res. Public Health 2018, 15, 2043
11. King, J. A. et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. Am. J. Gastroenterol. 115, 507–525 (2020).
12. Rintala A, Riikonen I, Toivonen A, et al. Early fecal microbiota composition in children who later develop celiac disease and associated autoimmunity. Scand J Gastroenterol 2018;53:403-409
13. Vriezinga SL, Auricchio R, Bravi E, et al. Randomized Feeding Intervention in Infants at High Risk for Celiac Disease. N Engl J Med 2014;371:1304-15.
14. Lionetti E, Castellaneta S, Francavilla R, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med 2014;371:1295-303.
15. Jansen MA, Tromp II, Kiefte-de Jong JC, Jaddoe VW, Hofman A, Escher JC, et al. Infant feeding and anti-tissue transglutaminase antibody concentrations in the Generation R Study. Am J Clin Nutr. (2014) 100:1095–101. doi: 10.3945/ajcn.114.090316.
16. Størdal K, White RA, Eggesbo M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132(5):e1202-9.
17. Andrén Aronsson CA, Lee HS, Liu E, et al. Age at gluten introduction and risk of celiac disease. Pediatrics 2015;135(2):239-45.
18. Andrén Aronsson C, Lee HS, Koletzko S, Uusitalo U, Yang J, Virtanen SM, et al. TEDDY Study Group. Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish Birth Cohort. Clin Gastroenterol Hepatol.(2016) 14:403–9. doi: 10.1016/j.cgh.2015.09.030

19. Mårild K, Kahrs CR, Tapia G, Stene LC, Størdal K. Infections and risk of celiac disease in childhood: a prospective nationwide cohort study. Am J Gastroenterol. (2015) 110:1475–84. doi: 10.1038/ajg.2015.287
20. Prediction models for celiac disease development in children from high-risk families: data from long term follow up of the PreventCD cohort. Meijer C, Auricchio R, Putter H, Castillejo G, Crespo P, Gyimesi J et al. Gastroenterology 2022, accepted
21. Leonard MM, Camhi S, Huedo-Medina TB, Fasano A. Celiac disease genomic, environmental, microbiome, and metabolomic (CDGEMM) study design: approach to the future of personalized prevention of celiac disease. Nutrients (2015) 7:9325–36.
22. Galipeau HJ, McCarville JL, Huebener S, Litwin O, Meisel M, Jabri B, et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. Am J Pathol. (2015) 185:2969–82.
23. Olivares M, Neef A, Castillejo G, Palma GD, Varea V, Capilla A, et al. The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. Gut. (2015) 64:406–17.
24. Olivares M, Walker AW, Capilla A, Benítez-Páez A, Palau F, Parkhill J, et al. Gut microbiota trajectory in early life may predict development of celiac disease. Microbiome (2018) 6:36.
25. Benitez-Paez A, Olivares M, Szajewska H, Piescik-Lech M, Polanco I, Castillejo G, et al. Breast-Milk Microbiota Linked to Celiac Disease Development in children: a pilot study from the PreventCD cohort. Front Microbiol 2020 Jun 23;11:1335.
26. Zafeiropoulou K, Nichols B, Mackinder M, et al. Alterations in intestinal microbiota of children with celiac disease at the time of diagnosis and on a gluten-free diet. Gastroenterology 2020 Dec;159(6):2039-2051
27. Fasano, A. Should we screen for coeliac disease? Yes. BMJ Clin Research 2009 339, 3592.
28. Ludvigsson J, Card T, Kaukinen K, Bai J, Zingone F, Sanders D, Murray J. Screening for celiac disease in the general population and in high-risk groups. United European Gastroenterol J. 2015 Apr;3(2):106-20
29. Evans KE, McAllister R, Sanders DS. Should we screen for coeliac disease? No. BMJ 2009 Sept 17;339:b3674
30. Meijer CR, Smit L, Overveld F, Mearin ML. Early diagnosis of coeliac disease by case-finding at the Preventive Youth Health Care Centres in the Netherlands (GLUTENSCREEN). Preliminary results. Abstract G-ePwP-011 World Congress of Pediatric Gastroenterology, Hepatology and Nutrition
31. Meijer CR, Ballintijn L, Mearin ML, Meij T, Smit L, Vries MC. Acceptability of active case-finding of celiac disease in the Netherlands. The GLUTENSCREEN study. Abstract G-eP-040, World Congress of Pediatric Gastroenterology, Hepatology and Nutrition
32. Rosén A, Sandström O, Carlsson A, et al. Usefulness of symptoms to screen for celiac disease. Pediatrics 2014 Feb 133(2):211–218
33. Jansen M, Zelm M, Groeneweg M, Jaddoe V, Dik W, Schreurs M, et al. The identification of celiac disease in asymptomatic children: the Generation R Study. J Gastroenterol. (2018) 53:377–86
34. Kiefte-de Jong JC, Jaddoe VW, Uitterlinden AG, et al. Levels of antibodies against tissue transglutaminase during pregnancy are associated with reduced fetal weight and birth weight. Gastroenterology. 2013;144:726–35.
35. Shamir R, Hernell O, Leshno M. Cost-effectiveness analysis of screening for celiac disease in the adult population. Med Decis Mak. 2006;26(3):282–93.

36. Park KT, Tsai R, Wang L, Khavari N, Bachrach L, Bass D. Cost-effectiveness of universal serologic screening to prevent nontraumatic hip and vertebral fractures in patients with celiac disease. Clin Gastroenterol Hepatol. 2013;11(6):645–53.
37. Mein SM, Ladabaum U. Serological testing for coeliac disease in patients with symptoms of irritable bowel syndrome: a cost-effectiveness analysis. Aliment Pharmacol Ther. 2004;19(11):1199–210.
38. Wilson JM, Jungner G: Principles and practice of screening for disease. Geneva: World Health Organisation; 1968.
39. CBO Richtlijn coeliakie en dermatitis herpetiformis. Haarlem: Nederlandse Vereniging van Maag-Darm-Leverartsen; http://www.diliguide.nl/document/2073/coeliakie-en-dermatitis-herpetiformis. html. 2008. Ref Type: Online Source.
40. Bai J. and Ciacci C., World Gastroentereology Organisation Global Guidelins: Celiac Disease February 2017. J Clin Gastroenterol 2017)
41. Biagi F, Corazza GR. First-degree relatives of celiac patients: are they at an increased risk of developing celiac disease? J Clin Gastroenterol 2009 Jan;43(1):3-4
42. Alhassan E, Yadav A, Kelly CP, Mukherjee R. Novel Nondietary Therapies for Celiac Disease. Cellular and Molecular Gastroenterology and Hepatology Vol. 8, No. 3. 2019
43. Meijer et al. Celiac Disease Prevention. Caroline Meijer, Raanan Shamir, Hania Szajewska, Luisa Mearin Front Pediatr. 2018; 6: 368. Published online 2018 Nov 30.
44. Kaukinen K, Sulkanen S, Maki M, Collin P. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. Eur J Gastroenterol Hepatol 2002;14:3
45. Leonard MM, Weir DC, DeGroote M, et al. Value of IgA tTG in Predicting Mucosal Recovery in Children With Celiac Disease on a Gluten-Free Diet. J Pediatr Gastroenterol Nutr 2017;64(2):286-91.
46. Wessels MMS, Te Lintelo M, Vriezinga SL, Putter H, Hopman EG, Mearin ML (2018) Assessment of dietary compliance in celiac children using a standardized dietary interview. Clin Nutr 37(3):1000–1004
47. Stefanolo JP, Tálamo M, Dodds S, et al. Real-World Gluten Exposure in Patients With Celiac Disease on Gluten-Free Diets, Determined From Gliadin Immunogenic Peptides in Urine and Fecal Samples. Clin Gastroenterol Hepatol. 2021;19(3):484-91.
48. Jeminiwa R, Hohmann L, Qian J, et al. Impact of E-health on medication adherence among patients with asthma: A systematic review and meta-analysis. Respir Med 2019;149(59-68).
49. Wessels M, Dolinsek J, Castillejo G, Donat E, Riznik P, Roca M et al (2021) Follow-up practices for children and adolescents with celiac disease: results of an international survey. Eur J Pediatr.
50. Blansky BA, Hintze ZJ, Alhassan E, Leichtner AM, Weir DC, Silvester JA (2019) Lack of Follow-up of Pediatric Patients With Celiac Disease. Clin Gastroenterol Hepatol 17(12):2603–2604.
51. Mearin ML, Agardh D, Antunes H, et al. ESPGHAN position paper on the management and follow-up of children and adolescents with coeliac disease. Submitted 2022

9

Algemene discussie en conclusie

Coeliakie is een immuun-gemedieerde aandoening, waarbij de HLA immunogenetische achtergrond (DQ2 en DQ8 heterodimeren) en de trigger uit de omgeving (gluten) aanleiding zijn om de ziekte te ontwikkelen. Beide factoren zijn noodzakelijk - maar niet voldoende - om coeliakie te ontwikkelen.

Coeliakie is een veel voorkomende ziekte met een breed spectrum aan intestinale en extra-intestinale symptomen en potentiële complicaties zoals osteoporose, andere auto-immuunziekten en zeldzame, maar ernstige maligniteiten. De prevalentie stijgt, wat vooral wordt toegeschreven aan de beschikbaarheid van betrouwbare screeningstests, de groeiende bekendheid van de ziekte onder hulpverleners en de identificatie van risicogroepen. De combinatie van deze factoren heeft wereldwijd tot een aanzienlijke stijging van het aantal coeliakie diagnoses geleid (1). Ondanks dat de kennis over de pathofysiologie, diagnose, behandeling en mogelijke therapeutische opties geleidelijk toeneemt, blijft het onduidelijk wie coeliakie ontwikkelt en wie niet. Tijdige diagnose en adequate behandeling en follow-up zijn belangrijke vragen op dit moment en aanleiding voor de studies die opgenomen zijn in dit proefschrift.

Volgens de richtlijn van de European Society of Gastroenterology, Hepatology and Nutrition (ESPGHAN) kan sinds 2012 de diagnose coeliakie bij kinderen gesteld worden zonder het nemen van dunne darm biopten als wordt voldaan aan strikte criteria: symptomen, bij herhaling waarden van antilichamen tegen weefseltransglutaminase (TGA)≥ 10 x de bovengrens van normaal, positieve anti-endomysium antilichamen (EMA) en de aanwezigheid van HLA-DQ2/DQ8 (2). Een prospectieve validatiestudie van deze benadering toonde positieve voorspellende waarden variërend van 99.63 (95% CI, 98.67-99.6) tot 100 (95% CI, 99.23-100) (3). In 2020 is de richtlijn voor de diagnose van coeliakie geactualiseerd (4). In hoofdstuk 2 van dit proefschrift laten resultaten van onze nationale prospectieve studie zien dat in Nederland, het jaar na de publicatie van de ‘non-biopsy’ benadering, de diagnose bij meer dan 75% van de kinderen correct werd gesteld. Om dit nog verder te verbeteren is het van belang dat de huisartsen en kinderartsen, die een essentiële rol spelen bij het vermoeden van de diagnose coeliakie en het aanvragen van de initiële serologische tests, rekening houden met de aanbevelingen van de ESPGHAN-richtlijn waarin geadviseerd wordt de diagnose te laten stellen door een kinderarts-MDL of een kinderarts die affiniteit heeft met coeliakie, om zowel over- als onder diagnose en de gevolgen daarvan te voorkomen. Door de voortdurend veranderende klinische presentatie, is het moeilijk om alle kinderen tijdig te diagnosticeren (5). Desondanks is in veel landen, waaronder in Nederland, een stijgende incidentie van coeliakie tijdens de kinderleeftijd gerapporteerd. Naast de combinatie van verschillende factoren, zoals de groeiende bekendheid van coeliakie onder zorgprofessionals, screening van hoogrisicogroepen en de beschikbaarheid van betrouwbare screeningstests (5, 6), wordt

echter ook gedacht aan een reële stijging van de incidentie (7). Vergelijkbare stijgingen zijn namelijk ook bij andere auto-immuunziekten en allergische aandoeningen bij kinderen gerapporteerd, zoals diabetes mellitus type 1, astma en allergische rhinitis (8-10). Begrijpen hoe de tolerantie voor gluten verloren gaat, is een fundamentele vraag die meer onderzoek vergt. Omgevingsfactoren zijn in verband gebracht met de toename van de ziekte-incidentie, zoals virale infecties tijdens de kindertijd of veranderingen in de darm-microbiota (samenstelling of metabolietproductie) (12).

In hoofdstuk 3 wordt een overzicht gegeven van de huidige kennis over de preventieve strategieën van coeliakie. Kennis van de pathofysiologie zou primaire preventieve strategieën mogelijk kunnen maken bij individuen die de genetische aanleg voor de ziekte hebben, maar tot nu toe is primaire preventie niet mogelijk. Voedingsadviezen voor zuigelingen zijn in dit verband prospectief bestudeerd en gebleken is dat noch het tijdstip van glutenintroductie, noch de duur van borstvoeding het risico op coeliakie beïnvloeden (13-17). Recente studies van geboortecohorten uit coeliakie-families suggereren dat de hoeveelheid gluten die vroeg in het leven wordt geconsumeerd, een (te voorkomen) risicofactor kan zijn voor de ontwikkeling van coeliakie (18-20). Maar voordat 'preventieve'-aanbevelingen over de hoeveelheden zouden kunnen worden gegeven, zou dit moeten worden bestudeerd in gerandomiseerde gecontroleerde interventiestudies.

De exacte microbiële samenstelling en de rol van dysbiose in de pathogenese van coeliakie zijn op dit moment nog niet bekend, maar een associatie tussen veranderingen in de darm-microbiota en de ontwikkeling van coeliakie is wel aangetoond. De resultaten van de CDGEMM-studie (Celiac Disease Genomic, Environmental, Microbiome, and Metabolomic) worden binnenkort verwacht. Deze zouden kunnen helpen de rol die de darmmicrobiota heeft bij de betrokkenheid in de pathogenese van coeliakie, te begrijpen (21). Dit zou kunnen leiden tot nieuwe preventieve mogelijkheden door toediening van probiotica. Echter, ook dan zullen vervolgstudies nodig zijn om beter te kunnen nagaan of probiotica de ziekte kunnen behandelen of voorkomen en om te bepalen welk type, dosis en duur van de probiotica moet worden gebruikt (22-26).

Zolang primaire preventie van coeliakie niet mogelijk is, lijkt het diagnosticeren van de ziekte in een vroeg stadium - secundaire preventie - de beste optie. Ondanks het stijgende aantal coeliakie diagnoses, blijft een aanzienlijk aantal mensen ongediagnosticeerd (11). De mogelijkheid en haalbaarheid van screeningstrategieën om onopgemerkte coeliakiepatiënten te identificeren moet worden onderzocht. Er zijn belangrijke vragen gerezen over wie op coeliakie moet worden getest en wanneer. Tijdige diagnose kan zowel door vroege opsporing (case-finding) als door bevolkingsonderzoek (mass-screening)

worden bereikt, hoewel beide methoden nog steeds controversieel zijn vanwege hun ethische implicaties (27-29).

In hoofdstuk 4 van dit proefschrift wordt ons nationale project, GLUTENSCREEN, naar vroege opsporing van coeliakie bij kinderen met coeliakie-gerelateerde symptomen, uit de algemene bevolking gepresenteerd. Dit is het eerste case-findingsproject naar coeliakie in Nederland met als doel aan te tonen dat case-finding haalbaar, efficiënt, kosteneffectief en goed geaccepteerd is door de algemene bevolking. In GLUTENSCREEN werd aan de ouders van alle kinderen van 1-4 jaar die het consultatiebureau in de regio Kennemerland voor een standaard controle bezochten, gevraagd naar coeliakie-gerelateerde symptomen (gestandaardiseerde vragenlijst). Als één of meer symptomen aanwezig waren, werd een sneltest op coeliakie-antilichamen uitgevoerd. Bij een positieve uitslag, hetgeen betekent dat coeliakie-antilichamen aanwezig zijn, werd het kind verwezen naar het Leids Universitair Medisch Centrum voor verdere diagnostiek. De resultaten van GLUTENSCREEN zijn boven verwachting: coeliakie werd bevestigd bij 1.8% van de geteste kinderen. Dat is meer dan de 1% die verwacht werd op basis van de huidige literatuur (30). Bovendien werd deze vroege opsporingsmethode naar coeliakie goed geaccepteerd door de ouders en de meerderheid van de betrokken zorgverleners (31). In de literatuur is echter bekend dat de voorspellende waarde van symptomen om coeliakie te identificeren beperkt is, aangezien symptomen die geassocieerd zijn met coeliakie even vaak voorkomen bij personen met als zonder de ziekte (32). In de database van GLUTENSCREEN zullen we nagaan of sommige symptomen beter onderscheid maken in de aanwezigheid van coeliakie en/of wat de optimale combinatie van symptomen is als indicator voor het testen op coeliakie. De belangrijkste beperking van deze vroege opsporingsmethode naar coeliakie is, zelfs wanneer goed uitgevoerd, dat de diagnose in subklinische kinderen gemist wordt (33). Een alternatief is een bevolkingsonderzoek om alle kinderen met coeliakie op te sporen, maar hiertegen is in 2017 bezwaar aangetekend door de Medisch Ethische Commissie Leiden-Den Haag-Delft (METC-LDD) en De Nederlandse Centrale Commissie Mensgebonden Onderzoek (CCMO). Opmerkelijk is echter dat 70% van de ouders uit de algemene Nederlandse bevolking een bevolkingsonderzoek naar coeliakie acceptabel vindt (31). Om de opsporingsmethode te optimaliseren, zou het toevoegen van de HLA-typering wenselijk zijn, omdat voor de ontwikkeling van coeliakie bepaalde genen noodzakelijk zijn (HLA-DQ2 en/of DQ8). Momenteel maakt HLA-typering geen deel uit van GLUTENSCREEN, omdat de bestaande techniek die DNA-extractie vereist, aanzienlijke nadelen heeft op locaties die niet beschikken over een laboratorium (zoals de consultatiebureaus). Deze aanpak zal ook het probleem van de asymptomatische kinderen niet oplossen. Zij zullen alleen door middel van een bevolkingsonderzoek naar coeliakie worden opgespoord. Een analyse uit de Rotterdamse ‘Generation R’-studie onder 6-jarige kinderen uit de algemene bevolking toonde in 1.3%

van de kinderen niet-gediagnosticeerde coeliakie (57/4442 gescreende kinderen was positief voor TGA). Dit ging gepaard met belangrijke gezondheidsproblemen, zoals een verminderde botdichtheid en een vertraagde gewichtsgroei (33). Daarnaast hadden kinderen van vrouwen met niet-herkende en dus onbehandelde coeliakie een verminderde foetale groei en een lager geboortegewicht (34). Tezamen met literatuur waaruit blijkt dat bevolkingsonderzoek naar coeliakie kosteneffectief is, wordt het tijd om de discussie over bevolkingsonderzoek naar coeliakie te heropenen (35-37). Na vele jaren is dit nog steeds een punt van discussie. De controverse over de vraag of de algemene bevolking een bevolkingsonderzoek naar coeliakie zou accepteren, lijkt te worden beantwoord door de voorlopige resultaten van GLUTENSCREEN: een overgrote meerderheid van de algemene bevolking (ongeveer 70%) vindt een bevolkingsonderzoek naar coeliakie acceptabel. De resultaten van PreventCD, een prospectieve, Europese, dieet-interventie studie onder zuigelingen met een genetische aanleg uit coeliakie-families, hebben ook waardevolle informatie opgeleverd over het natuurlijke beloop van coeliakie. Zowel bij kinderen uit risicogroepen (coeliakie-families) als in de algemene bevolking ontwikkelt coeliakie zich zeer vroeg. Met het verkrijgen van deze nieuwe inzichten lijkt het tijd om de discussie “is het tijd voor een bevolkingsonderzoek naar coeliakie” te heropenen, aangezien aan alle tien criteria voor bevolkingsonderzoek, opgesteld door Wilson en Jungner, is voldaan (38). Testen op coeliakie bij asymptomatische kinderen zal inzicht geven in de werkelijke prevalentie van coeliakie en zal een tijdige diagnose mogelijk maken voor alle kinderen met coeliakie. Het zal een belangrijke stap in de goede richting zijn voor de preventieve zorg in Nederland.

Screening op coeliakie van hoog-risicogroepen, zoals eerstegraad familieleden van coeliakie -patiënten en patiënten met auto-immuunziekten, wordt al aanbevolen door zowel de Nederlandse als de meeste internationale richtlijnen (2, 39, 40). Er is echter weinig informatie bekend over de verbetering van symptomen na behandeling bij kinderen die door vroege opsporing gediagnosticeerd zijn met coeliakie. Ongeveer de helft van deze kinderen heeft klachten op het moment van diagnose. In onze PreventCD studie zijn kinderen vanaf de geboorte gevolgd op de ontwikkeling van coeliakie door middel van een gestandaardiseerde vragenlijst over hun gezondheid (gerapporteerd door de ouders), groei en coeliakie-antilichamen. Gegevens van deze studie zijn geanalyseerd om te beoordelen of kinderen uit coeliakiefamilies baat hebben bij het vroegtijdig opsporen en behandelen van de ziekte. Deze gegevens tonen aan dat de meeste symptomen significant verbeteren na behandeling met een glutenvrij dieet en dat vroege opsporing ook klinisch voordeel biedt (manuscript in voorbereiding).

Dat kinderen uit coeliakiefamilies een hoger risico hebben om de ziekte te ontwikkelen, is algemeen bekend, maar in hoofdstuk 5 laten de resultaten van het PreventCD-cohort

een significant hoger risico zien gedurende de eerste levensjaren dan voorheen gerapporteerd (1, 41). Tot voor kort werd het risico op coeliakie voor eerste graad familieleden van coeliakiepatiënten vastgesteld op 5%-10%. Onze gegevens tonen echter dat op de leeftijd van acht jaar de kans op coeliakie maar liefst 17% is. Dit benadrukt het belang van vroege opsporing en diagnose nog meer. Gegevens over de frequentie van screening en de leeftijd waarop deze moet worden uitgevoerd, ontbreken vooralsnog. Verschillende factoren beïnvloeden het risico op de ontwikkeling van coeliakie. Zo ontwikkelt coeliakie zich al op zeer jonge leeftijd (gemiddelde leeftijd 4.3 jaar), komt het significant vaker voor bij meisjes ($p=0.005$) en bij HLA-DQ2 homozygote personen ($p<0.001$). Gebaseerd op deze factoren, en de huidige leeftijd, hebben wij predictiemodellen voor de ontwikkeling van coeliakie gemaakt. Hiermee kan een geïndividualiseerd screeningsadvies gegeven worden aan kinderen uit coeliakie-families (hoofdstuk 5). Door PreventCD en GLUTENSCREEN weten we nu dus dat het natuurlijke beloop van coeliakie een zeer vroege ontwikkeling in het leven kent. Het zal nog geëvalueerd moeten worden of de predictiemodellen en de daaruit voortvloeiende screeningsadviezen ook toepasbaar zijn op kinderen uit de algemene bevolking.

Een tijdige diagnose van deze chronische ziekte is gunstig voor (a)symptomatische kinderen, maar de follow-up na de diagnose is even belangrijk. De enige behandeling voor coeliakie is een levenslang strikt glutenvrij dieet. Dit is niet altijd even makkelijk vol te houden, omdat gluten aanwezig zijn in de meeste verwerkte voedingsmiddelen. Daarnaast kunnen dieetbeperkingen een nadelige invloed op de kwaliteit van leven hebben. Bovendien zijn glutenvrije voedingsmiddelen niet overal verkrijgbaar, zijn ze duurder en minder smakelijk, wat kan leiden tot een lage therapietrouw. Er worden momenteel verschillende studies uitgevoerd om niet-diëtetaire behandelingen te onderzoeken als mogelijke opties voor tertiaire preventie (42).

Goede therapietrouw van het glutenvrij dieet vermindert de complicaties van coeliakie en kan worden beschouwd als een tertiaire preventieve maatregel (43). Veelal wordt tijdens de follow-up van coeliakiepatiënten TGA bepaald als maat voor darmherstel. Maar de resultaten van de coeliakie-antistoffen correleren niet goed met dieettrouw (44). Ondanks het ontbreken van een gouden standaard voor het beoordelen van de therapietrouw, wordt een evaluatie van het glutenvrij dieet door een ervaren diëtist beschouwd als de beste methode. Dit kent echter ook nadelen: het is tijdrovend en vereist deskundig personeel dat niet altijd beschikbaar is. Alternatieven als korte dieetvragenlijsten en bepalingen van TGA slagen er echter niet in dieetfouten te detecteren bij kinderen en adolescenten met coeliakie. Daarmee zijn deze methoden niet gevoelig genoeg om alle patiënten die gluten consumeren te identificeren (45). Om de dieettrouw bij kinderen en adolescenten met coeliakie te beoordelen, is een dieetvragenlijst ontwik-

keld en gevalideerd (46). Ook is een andere methode ontwikkeld om gluteninname op te sporen, gluten-immunogene-peptiden (GIP) (47), maar deze test wordt nog niet gebruikt in de standaard klinische zorg. In hoofdstuk 6 tonen de resultaten van onze klinische studie dat de combinatie van de dieetvragenlijst en de GIP-test in urine, de meest effectieve methode is in het opsporen van (on)opzettelijke glutenfouten. Beide testen, evenals de TGA-bepaling, hebben echter hun beperkingen bij het monitoren van dieettrouw. Omdat de GIP-test gluten detecteert die slechts enkele dagen voor de test zijn ingenomen, kan glutenconsumptie vóór die tijd onopgemerkt blijven. Zoals gepresenteerd in onze studie, kan GIP-bepaling nuttig zijn in specifieke klinische situaties om (on)opzettelijke gluteninname uit te sluiten, bijvoorbeeld bij kinderen 1. bij wie recent de diagnose coeliakie is gesteld en die vertrouwd raken met het glutenvrij dieet, 2. die symptomen rapporteren met een negatieve TGA en geen dieetfouten hebben gerapporteerd op de dieetvragenlijst, 3. met (persisterende) verhoogde of zeer langzame normalisatie van TGA ondanks dat zij geen fouten op de dieetvragenlijst hebben aangegeven en 4. met vermoedelijke opzettelijke gluteninname. In tegenstelling tot de momenteel bruikbare en betrouwbare test voor screening en diagnose, is er behoefte aan een klinisch bruikbare biomarker die kan helpen bij de monitoring van de ziekte over een langere periode.

De standaard medische zorg voor coeliakiepatiënten bestaat uit regelmatige doktersbezoeken. De beperkte tijd die wordt uitgetrokken voor een poliklinisch consult verhindert gewoonlijk een uitgebreide beoordeling van de kwaliteit van leven en de therapietrouw van de patiënt (4). Daarom moeten andere mogelijkheden buiten het ziekenhuis worden overwogen. Zelfmanagement heeft gunstige effecten laten zien op de gezondheidszorg bij andere chronische ziekten (48). E-Health kan een belangrijke rol spelen bij de ondersteuning van patiënten bij zelfmanagement, aangezien internet en technologie gebruikers gemakkelijk en snel kunnen bereiken. E-Health wordt gedefinieerd als diensten en informatie op het gebied van gezondheidszorg die elektronisch via het internet en aanverwante technologieën worden geleverd of verbeterd. In hoofdstuk 7 worden de resultaten gepresenteerd van een systematisch review naar het gebruik en de effecten van elektronische gezondheidszorgtechnologieën bij de follow-up van coeliakiepatiënten. De meerderheid van de patiënten is tevreden over E-Health. Het gebruik kan effectief zijn bij het verbeteren van zorg in specifieke aspecten, de kwaliteit van leven, therapietrouw en kennis over coeliakie en het glutenvrij dieet.

Tijdens de controles van kinderen en adolescenten met coeliakie is het van belang om symptomen, gewicht/lengte en kwaliteit van leven te beoordelen en complicaties te voorkomen. Ook wordt aandacht besteed aan ziekte-educatie, sociale steun en het motiveren van therapietrouw van het kind en zijn familie, waarbij het belang van goede voeding wordt benadrukt. Momenteel is de follow-up van kinderen met coeliakie niet

gestandaardiseerd en grotendeels gebaseerd op de mening van deskundigen, wat leidt tot aanzienlijke verschillen in follow-up tussen landen en zelfs regionaal binnen landen die hetzelfde gezondheidszorgsysteem toepassen (49-50). Door een internationale samenwerking van coeliakie-geïnteresseerden is ondertussen de ESPGHAN position-paper voor de follow up van coeliakie opgesteld. Op basis van beschikbare literatuur zijn aanbevelingen geformuleerd voor een meer gestructureerde follow-up van kinderen met coeliakie (51). Laten we hopen dat deze aanbevelingen net zo snel en efficiënt worden opgevolgd als de ESPGHAN-richtlijn voor de diagnose van coeliakie.

TOEKOMSTPERSPECTIEVEN

Aangezien primaire preventie van coeliakie een zeer aantrekkelijke, maar nog niet gerealiseerde doelstelling is, moet de nadruk voorlopig liggen op het bevorderen van een snelle diagnose en behandeling bij (a)symptomatische kinderen en adolescenten. De voorlopige resultaten van GLUTENSCREEN laten zien dat vroege opsporing op de consultatiebureaus een aanzienlijk deel van de anders onopgemerkte kinderen opspoort en de tijd tot diagnose verkort. Daarnaast geeft het ons informatie over de kosteneffectiviteit en aanvaardbaarheid van een vroege coeliakie-diagnose bij symptomatische kinderen. Op basis van deze positieve resultaten hebben de consultatiebureaus in de regio Kennemerland besloten de vroege opsporing naar coeliakie in hun reguliere zorg te implementeren. Gestreefd wordt om deze opsporingsmethode te implementeren bij alle andere consultatiebureaus in Nederland. Om de doelpopulatie van vroege opsporing naar coeliakie te optimaliseren, is financiering aangevraagd voor de ontwikkeling van een nieuwe test om HLA-typering uit te voeren in bloeddruppels (op filterpapier, zoals bij de hielprikscreening) die worden verkregen bij de sneltest op coeliakie-antilichamen op het consultatiebureau. Deze aanpak zal de succesvolle vroege opsporingsmethode naar coeliakie effectiever maken en daarmee de belasting van de HLA-DQ2/8 negatieve kinderen verminderen. In de tussentijd blijven we zoeken naar mogelijkheden voor primaire preventie.

REFERENCES

1. Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, et al (2018) Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Volume 16, Issue 6, pp 823–836.
2. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012;54(1):136–60
3. Werkstetter KJ, Korponay-Szabó IR, Popp A, et al. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology. 2017;153(4):924-935.
4. Husby S, Koletzko S, Korponay-Szabó IR et al. European Society Pediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 70(1):141-156.
5. Meijer CR, Schweizer JJ, Peeters A, Putter H, Mearin ML. Efficient implementation of the 'non-biopsy approach' for the diagnosis of childhood celiac disease in the Netherlands: a national prospective evaluation 2010–2013. Eur J Pediatr. 2021; 180(8): 2485–2492.
6. Riznik P, De Leo L, Dolinsek J, Judit Gyimesi J, Martina Klemenak M, Berthold Koletzko B, et al. Clinical Presentation in Children With Coeliac Disease in Central Europe. J Pediatr Gastroenterol Nutr. 2021 Apr 1;72(4):546-551.
7. Almallouhi E, King KS, Patel B, Wi C, Juhn YJ, Murray JA, Absah I. Increasing incidence and altered presentation in a population-based study of pediatric celiac disease in North America. J Pediatr Gastroenterol Nutr 2017; 65:432–437
8. Lipman TH, et al. Increasing incidence of type 1 diabetes in youth: twenty years of the Philadelphia Pediatric Diabetes Registry. Diabetes care. 2013; 36(6):1597–603.
9. Lindfors, K. et al. Metagenomics of the faecal virome indicate a cumulative effect of enterovirus and gluten amount on the risk of coeliac disease autoimmunity in genetically at risk children: the TEDDY study. Gut 69, 1416–1422 (2020).
10. Loh W, Tang MLK. The Epidemiology of Food Allergy in the Global Context. Int. J. Environ. Res. Public Health 2018, 15, 2043
11. King, J. A. et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. Am. J. Gastroenterol. 115, 507–525 (2020).
12. Rintala A, Riikonen I, Toivonen A, et al. Early fecal microbiota composition in children who later develop celiac disease and associated autoimmunity. Scand J Gastroenterol 2018;53:403-409
13. Vriezinga SL, Auricchio R, Bravi E, et al. Randomized Feeding Intervention in Infants at High Risk for Celiac Disease. N Engl J Med 2014;371:1304-15.
14. Lionetti E, Castellaneta S, Francavilla R, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med 2014;371:1295-303.
15. Jansen MA, Tromp II, Kiefte-de Jong JC, Jaddoe VW, Hofman A, Escher JC, et al. Infant feeding and anti-tissue transglutaminase antibody concentrations in the Generation R Study. Am J Clin Nutr. (2014) 100:1095–101. doi: 10.3945/ajcn.114.090316.
16. Størdal K, White RA, Eggesbo M. Early feeding and risk of celiac disease in a prospective birth cohort. Pediatrics 2013;132(5):e1202-9.
17. Andrén Aronsson CA, Lee HS, Liu E, et al. Age at gluten introduction and risk of celiac disease. Pediatrics 2015;135(2):239-45.
18. Andrén Aronsson C, Lee HS, Koletzko S, Uusitalo U, Yang J, Virtanen SM, et al. TEDDY Study Group. Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish Birth Cohort. Clin Gastroenterol Hepatol.(2016) 14:403–9. doi: 10.1016/j.cgh.2015.09.030

19. Mårild K, Kahrs CR, Tapia G, Stene LC, Størdal K. Infections and risk of celiac disease in childhood: a prospective nationwide cohort study. Am J Gastroenterol. (2015) 110:1475–84. doi: 10.1038/ajg.2015.287
20. Prediction models for celiac disease development in children from high-risk families: data from long term follow up of the PreventCD cohort. Meijer C, Auricchio R, Putter H, Castillejo G, Crespo P, Gyimesi J et al. Gastroenterology 2022, accepted
21. Leonard MM, Camhi S, Huedo-Medina TB, Fasano A. Celiac disease genomic, environmental, microbiome, and metabolomic (CDGEMM) study design: approach to the future of personalized prevention of celiac disease. Nutrients (2015) 7:9325–36.
22. Galipeau HJ, McCarville JL, Huebener S, Litwin O, Meisel M, Jabri B, et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. Am J Pathol. (2015) 185:2969–82.
23. Olivares M, Neef A, Castillejo G, Palma GD, Varea V, Capilla A, et al. The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. Gut. (2015) 64:406–17.
24. Olivares M, Walker AW, Capilla A, Benítez-Páez A, Palau F, Parkhill J, et al. Gut microbiota trajectory in early life may predict development of celiac disease. Microbiome (2018) 6:36.
25. Benitez-Paez A, Olivares M, Szajewska H, Piescik-Lech M, Polanco I, Castillejo G, et al. Breast-Milk Microbiota Linked to Celiac Disease Development in children: a pilot study from the PreventCD cohort. Front Microbiol 2020 Jun 23;11:1335.
26. Zafeiropoulou K, Nichols B, Mackinder M, et al. Alterations in intestinal microbiota of children with celiac disease at the time of diagnosis and on a gluten-free diet. Gastroenterology 2020 Dec;159(6):2039-2051
27. Fasano, A. Should we screen for coeliac disease? Yes. BMJ Clin Research 2009 339, 3592.
28. Ludvigsson J, Card T, Kaukinen K, Bai J, Zingone F, Sanders D, Murray J. Screening for celiac disease in the general population and in high-risk groups. United European Gastroenterol J. 2015 Apr;3(2):106-20
29. Evans KE, McAllister R, Sanders DS. Should we screen for coeliac disease? No. BMJ 2009 Sept 17;339:b3674
30. Meijer CR, Smit L, Overveld F, Mearin ML. Early diagnosis of coeliac disease by case-finding at the Preventive Youth Health Care Centres in the Netherlands (GLUTENSCREEN). Preliminary results. Abstract G-ePwP-011 World Congress of Pediatric Gastroenterology, Hepatology and Nutrition
31. Meijer CR, Ballintijn L, Mearin ML, Meij T, Smit L, Vries MC. Acceptability of active case-finding of celiac disease in the Netherlands. The GLUTENSCREEN study. Abstract G-eP-040, World Congress of Pediatric Gastroenterology, Hepatology and Nutrition
32. Rosén A, Sandström O, Carlsson A, et al. Usefulness of symptoms to screen for celiac disease. Pediatrics 2014 Feb 133(2):211–218
33. Jansen M, Zelm M, Groeneweg M, Jaddoe V, Dik W, Schreurs M, et al. The identification of celiac disease in asymptomatic children: the Generation R Study. J Gastroenterol. (2018) 53:377–86
34. Kiefte-de Jong JC, Jaddoe VW, Uitterlinden AG, et al. Levels of antibodies against tissue transglutaminase during pregnancy are associated with reduced fetal weight and birth weight. Gastroenterology. 2013;144:726–35.
35. Shamir R, Hernell O, Leshno M. Cost-effectiveness analysis of screening for celiac disease in the adult population. Med Decis Mak. 2006;26(3):282–93.

36. Park KT, Tsai R, Wang L, Khavari N, Bachrach L, Bass D. Cost-effectiveness of universal serologic screening to prevent nontraumatic hip and vertebral fractures in patients with celiac disease. Clin Gastroenterol Hepatol. 2013;11(6):645–53.
37. Mein SM, Ladabaum U. Serological testing for coeliac disease in patients with symptoms of irritable bowel syndrome: a cost-effectiveness analysis. Aliment Pharmacol Ther. 2004;19(11):1199–210.
38. Wilson JM, Jungner G: Principles and practice of screening for disease. Geneva: World Health Organisation; 1968.
39. CBO Richtlijn coeliakie en dermatitis herpetiformis. Haarlem: Nederlandse Vereniging van Maag-Darm-Leverartsen; http://www.diliguide.nl/document/2073/coeliakie-en-dermatitis-herpetiformis. html. 2008. Ref Type: Online Source.
40. Bai J. and Ciacci C., World Gastroentereology Organisation Global Guidelins: Celiac Disease February 2017. J Clin Gastroenterol 2017)
41. Biagi F, Corazza GR. First-degree relatives of celiac patients: are they at an increased risk of developing celiac disease? J Clin Gastroenterol 2009 Jan;43(1):3-4
42. Alhassan E, Yadav A, Kelly CP, Mukherjee R. Novel Nondietary Therapies for Celiac Disease. Cellular and Molecular Gastroenterology and Hepatology Vol. 8, No. 3. 2019
43. Meijer et al. Celiac Disease Prevention. Caroline Meijer, Raanan Shamir, Hania Szajewska, Luisa Mearin Front Pediatr. 2018; 6: 368. Published online 2018 Nov 30.
44. Kaukinen K, Sulkanen S, Maki M, Collin P. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. Eur J Gastroenterol Hepatol 2002;14:3
45. Leonard MM, Weir DC, DeGroote M, et al. Value of IgA tTG in Predicting Mucosal Recovery in Children With Celiac Disease on a Gluten-Free Diet. J Pediatr Gastroenterol Nutr 2017;64(2):286-91.
46. Wessels MMS, Te Lintelo M, Vriezinga SL, Putter H, Hopman EG, Mearin ML (2018) Assessment of dietary compliance in celiac children using a standardized dietary interview. Clin Nutr 37(3):1000–1004
47. Stefanolo JP, Tálamo M, Dodds S, et al. Real-World Gluten Exposure in Patients With Celiac Disease on Gluten-Free Diets, Determined From Gliadin Immunogenic Peptides in Urine and Fecal Samples. Clin Gastroenterol Hepatol. 2021;19(3):484-91.
48. Jeminiwa R, Hohmann L, Qian J, et al. Impact of E-health on medication adherence among patients with asthma: A systematic review and meta-analysis. Respir Med 2019;149(59-68).
49. Wessels M, Dolinsek J, Castillejo G, Donat E, Riznik P, Roca M et al (2021) Follow-up practices for children and adolescents with celiac disease: results of an international survey. Eur J Pediatr.
50. Blansky BA, Hintze ZJ, Alhassan E, Leichtner AM, Weir DC, Silvester JA (2019) Lack of Follow-up of Pediatric Patients With Celiac Disease. Clin Gastroenterol Hepatol 17(12):2603–2604.
51. Mearin ML, Agardh D, Antunes H, et al. ESPGHAN position paper on the management and follow-up of children and adolescents with coeliac disease. Submitted 2022

A

ABBREVIATIONS

AGA	=	Anti-gliadin antibodies;
AIC	=	Akaike Information Criterion;
CBS	=	Central Bureau of Statistics;
CD	=	Celiac disease;
CDA	=	Celiac disease autoimmunity;
CI	=	Confidence interval;
CME-LUMC	=	Medical Ethics Committee of the Leiden University Medical Centre (later called METC-LDD = Medical Ethics Committee- Leiden Den Haag Delft);
CON	=	Cut-off of normality;
DPSU	=	Dutch Pediatric Surveillance Unit. In Dutch: Nederlands Signalerings Centrum Kindergeneeskunde, NSCK;
DSP	=	Dutch Society of Paediatrics;
DQ	=	Dietary questionnaire;
E-health	=	Electronic-health technologies;
ELIA	=	Enzyme-linked immunoassay;
EMA	=	Endomysium antibodies;
ESPGHAN	=	European Society for Pediatric Gastroenterology Hepatology and Nutrition;
FDR	=	First degree relative;
GFD	=	Gluten free diet;
GIP	=	Gluten immunogenic peptides;
HLA	=	Human leukocyte antigen;
IgA	=	Immunoglobulin A;
IQR	=	Inter-quartile range;
LUMC	=	Leiden University Medical Centre;
MeSH	=	Medical Subject Headings;
NCV	=	Dutch Coeliac Society
NEJM	=	New England Journal of Medicine;
PALGA	=	Pathologisch Landelijk Geautomatiseerd Archief= National Database of Pathology;
POCT	=	Point of contact test;
PREVENTCD	=	Multicenter European study funded by the European Commission FP-6-2005-FOOD-4B; Proposal/Contract no 036383: Influence of the dietary history in the prevention of celiac disease: possibilities of induction of tolerance for gluten in genetic predisposed children;

PRISMA	=	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
QoL	=	Quality of life;
RCT	=	Randomized-control trial;
SAP	=	Statistical analysis plan;
SD	=	Standard deviation;
SNPs	=	Single-nucleotide polymorphisms;
SQL	=	Structured Query Language;
tTG / TG2A / TGA	=	Anti-tissue transglutaminase antibodies
ULN	=	Upper limit of normal;
YHCC	=	Youth Health Care Centres

LIST OF PUBLICATIONS

Prediction models for celiac disease development in children from high-risk families: Data from the PreventCD cohort. Meijer CR, Auricchio R, Castillejo G, Crespo Escobar P, Gyimesi J, Hartman C, Kolacek S, Koletzko S, Korponay-Szabo IR, Martinez Ojinaga Nodal E, Pieścik-Lech M, Polanco I, Ribes Koninckx C, Shamir R, Szajewska H, Szillat P, Troncone R, Werkstetter K, Mearin LM. Gastroenterology 2022 Aug;163(2):426-436

Association in clinical practice between gluten-intake and gluten immunogenic peptides in celiac children. Caroline R. Meijer, Jaap Bakker, Anneloes Boers, Sophie Jansen, Zeliha Mengi, M. Luisa Mearin. Gastro Hep Advances 2022; 1:652–65

ESPGHAN position paper on the follow-up and management of coeliac disease in children and adolescents. Mearin ML, Agardh D, Antunes H, Al-toma A, Auricchio R, Castillejo G, Catassi C, Ciacci C, Discepolo V, Dolinsek J, Donat E, Gillett P, Guandalini S, Husby S, Koletzko S, Koltai T, Korponay-Szabó IR, Kurppa K, Lionetti E, Mårild K, Martinez Ojinaga E, Meijer C, Monachesi C, Polanco I, Popp A, Roca M, Rodriguez Herrera A, Shamir R, Stordal K, Troncone R, Valitutti F, Vreugdenhill A, Wessels M, Whiting P on behalf of the ESPGHAN Special Interest Group on Celiac Disease** J Pediatr Gastroenterol Nutr. 2022 Sep 1;75(3):369-386

Review on pediatric coeliac disease from a clinical perspective European Journal of Pediatrics. Wessels M, Auricchio R, Dolinsek J, Donat E, Gillett P, Marild K, Meijer C, Popp A, Mearin ML. European Journal of Pediatrics 2022, May;181(5):1785-1795.

Utilization and effectiveness of eHealth technology in the follow-up of celiac disease: A systematic review. Alice Loft Månsson, Caroline Meijer-Boekel, Karl Mårild. JPGN 2022 Jun1;74(6):812-818

Circulating miRNAs as potential biomarkers for celiac disease development. Ineke Luise Tan, Rodrigo Coutinho de Almeida, Rutger Modderman, Anna Stachurska, Jackie Dekens, Donatella Barisani, Caroline Renee Meijer-Boekel, María Roca, Eva Martinez-Ojinaga, Raanan Shamir, Renata Auricchio, Ilma R Korponay-Szabó, Gemma Castillejo, Hania Szajewska, Sibylle Koletzko, Alexandra Zhernakova, Vinod Kumar, Yang Li, Marijn C Visschedijk, Rinse K Weersma, Riccardo Troncone, M Luisa Mearin, Cisca Wijmenga, Iris Jonkers, Sebo Withoff. Front Immunol. 2021 Dec 7;12:734763

Efficient implementation of the 'non-biopsy approach' for the diagnosis of childhood celiac disease in the Netherlands: a national prospective evaluation 2010–2013.

Caroline R. Meijer, Joachim J. Schweizer, Anne Peeters, Hein Putter, M. Luisa Mearin. Eur J Pediatr. 2021; 180(8): 2485–2492.

Early diagnosis of coeliac disease in the Preventive Youth Health Care Centres in the Netherlands: study protocol of a case finding study (GLUTENSCREEN). Caroline Meijer-Boekel, M.Elske van den Akker, Leti van Bodegom, Johanna Escher, Nan van Geloven, Floris van Overveld, Edmond H H.M Rings, Lucy Smit, Martine C. de Vries, M. Luisa Mearin. BMJ Paediatr Open. 2021; 5(1)

Growth rate of coeliac children is compromised before the onset of the disease. Auricchio R, Stellato P, Bruzzese D, Cielo D, Chiurazzi A, Galatola M, Castilljeo G,Escobar PC, Gyimesi J, Hartman C, Kolacek S, Koletzko S, Korponay-Szabo I, Mearin ML, Meijer C, Piescik-Lech M, Polanco I, Ribes-Koninckx C, Shamir R, Szajewska H, Troncone R, Greco L. Arch Dis Child. 2020 Oct;105(10):964-968.

Breast-Milk Microbiota Linked to Celiac Disease Development in Children: A pilot study from the PreventCD cohort. Alfonso Benítez-Páez, Marta Olivares, Hania Szajewska, Małgorzata Pieścik-Lech, Isabel Polanco, Gemma Castillejo, Merce Nuñez, Carmen Ribes-Koninckx, Ilma R. Korponay-Szabó, Sibylle Koletzko, Caroline R. Meijer, M. Luisa Mearin, Yolanda Sanz. Front Microbiol. 2020; 11: 1335. Published online 2020 Jun 23.

Intestinal anti-transglutaminase IgA deposits in children at risk for coeliac disease: data from the Prevent CD study. Short title: Intestinal anti-TG2 deposits in at-risk children. Melissa Borrelli, Mariantonia Maglio, Ilma R Korponay-Szabò, M. Luisa Mearin, Caroline Meijer, Hagit Niv-Drori, Carmen Ribes-Koninckx, María Roca, Raanan Shamir, Riccardo Troncone and Renata Auricchio. Clin Exp Immunol. 2018 Mar;191(3):311-317.

Celiac Disease Prevention. Caroline Meijer, Raanan Shamir, Hania Szajewska, Luisa Mearin Front Pediatr. 2018; 6: 368. Published online 2018 Nov 30.

Does Infant Feeding Modulate the Manifestation of Celiac Disease and Type Diabetes? C.R.Meijer, V. Discepolo, R. Troncone, M.L.Mearin. Curr Opin Clin Nutr Metab Care. 2017 May;20(3):222-226.

Children from coeliac families benefit from early diagnosis and treatment: an analysis of the PreventCD cohort. Meijer CR, Auricchio R, Castillejo, Crespo Escobar P, Gyimesi J, Hartman C, Kolacek S, Koletzko S, Korponay-Szabo IR, Martinez Ojinaga Nodal E, Pieścik-Lech M, Polanco I, Ribes Koninckx C, Shamir R, Szajewska H, Szillat P, Troncone R, Werkstetter K, Mearin LM. In preparation.

Szajewska H, Shamir R, Chmielewska A, Stróżyk A, Zalewski BM, Auricchio R, Koletzko S, Korponay-Szabo IR, Mearin ML, Meijer CR, Ribes-Koninckx C, Troncone R. Early feeding practices and celiac disease prevention. Protocol for an updated and revised systematic review and meta-analysis. Nutrients. 2022 Feb 28;14(5):1040.

DANKWOORD

Dit proefschrift is het resultaat van een samenwerking met velen, aan wie ik dank verschuldigd ben. Het is en was fantastisch om te mogen leren van zoveel mensen. Mensen die mij gevormd hebben op zowel medisch inhoudelijk, wetenschappelijk als menselijk vlak. Graag wil ik iedereen bedanken die direct of indirect heeft bijgedragen aan de totstandkoming van dit proefschrift. En een aantal personen wil ik graag in het bijzonder bedanken.

Als eerste en belangrijkste wil ik de (ouders/verzorgers van) kinderen die hebben willen deelnemen aan onder andere PreventCD of GLUTENSCREEN, bedanken. Zonder jullie hulp zou de zorg voor coeliakie-patiënten niet verbeteren.

Prof. dr. M.L Mearin, lieve Luisa, jij gaf mij het volste vertrouwen en de kans om zowel een fellowship als een promotietraject te doen. Ik bewonder en waardeer jouw kennis, enthousiasme en doorzettingsvermogen.

Prof. dr. E.H.H.M. Rings, beste Edmond, ondanks al jouw drukke werkzaamheden, had je altijd tijd voor een kritische blik. Jouw adviezen zijn erg waardevol.

Graag wil ik professor Gerd Bouma en professor Tom Huizinga bedanken voor het plaatsnemen in de promotiecommissie. Tevens wil ik de leden van de oppositiecommissie bedanken voor hun bereidheid om met mij van gedachten te wisselen over dit proefschrift.

The PreventCD group for giving me the opportunity to work with, and learn from a group so well established in the field of celiac disease research.

Lieve ‘ES’-kinderartsen: wij hebben een geweldig team en individueel zijn jullie toppers! Veel dank voor alle motiverende gesprekken, stimulerende woorden en plezierige lunches en uitjes. Graag wil ik ook de doktersassistenten, arts-assistenten, verpleegkundigen, student-assistenten en diëtisten van het LUMC en de Jeugdgezondheidszorg Kennemerland bedanken voor al jullie inzet en gezelligheid. Speciale dank gaat uit naar Joachim, Lucy, Inge, Wendy en Yvonne.

Collega’s van de kinder-MDL van het ErasmusMC (ook Pauline de Bruyne!) dank voor al jullie support zowel op wetenschappelijk als klinisch gebied. Jullie zijn fantastische, warme mensen met een kritische blik.

Ram Sukhai, bedankt voor de kans en het vertrouwen dat u mij gaf om de opleiding kindergeneeskunde te doen.

Vrienden van vroeger en nu, “de Rotterdamse arbeiders”, luizen-, bakfiets-, en schoolpleinmoeders (en vaders), maar in het bijzonder wil ik Willemijn bedanken. Een vriendschap vanaf de kleuterschool, lieve Wil, jij bent de zus die ik altijd al wilde hebben.

Als laatste wil ik mijn familie bedanken. Allereerst onze rots in de branding, Wilma. Zoals je ziet hoor je bij de familie. Inmiddels 7 jaar de vaste oppas van onze kinderen, maar ook ik kan niet zonder jou. Ik ben jaloers op jouw kookkunsten, creativiteit en strijk/vouwtechniek. Zonder jou hadden wij het thuis niet kunnen bolwerken.

Dan mijn schoonouders, Elzeline en Piet, dank voor jullie waardering en interesse in mijn werkzaamheden. Helaas heeft Elzeline dit proefschrift niet meer mogen meemaken. Ik weet zeker dat ze apetrots zou zijn geweest. Piet, ik ben blij dat jij, na een moeilijke periode, aanwezig kunt zijn bij de verdediging van dit proefschrift. Piet-Hein, Jacqueline, Jack en Julie: dank voor jullie betrokkenheid en steun in de afgelopen jaren.

Lieve oom Hans en oom PP, daar staat jullie kleine nichtje. Zo leuk dat jullie altijd tijd maken om bij de familie-festiviteiten te zijn, zo ook nu. Bedankt voor de grenzeloze steun en support.

Mijn lieve broers, Robert, Hans en Pieter. Jullie vragen je weleens af wat jullie kleine zusje toch allemaal doet in de avonduren achter de computer. Hopelijk begrijpen jullie het nu er eindelijk een boekje is. Ik ben blij dat jullie bij belangrijke momenten altijd aanwezig zijn. Dank dat jullie mijn broers zijn en dank voor de schoonzussen, Marlies, Esther, Joëlle en mijn neefje Duco en nichtjes Vesper, Lauren, Rosa en Kiki.

Speciaal voor jou, lieve Rosa, zal ik onderzoek blijven doen naar deze ziekte, nu jij in de laatste maanden van mijn promotieonderzoek bent gediagnosticeerd met coeliakie (door case-finding!).

Mijn ouders, lieve papa en mama, wat ben ik blij dat jullie deze mijlpaal allebei mogen meemaken. Ik kan jullie niet genoeg bedanken voor alle mogelijkheden die jullie mij hebben geboden. Zonder jullie onvoorwaardelijke liefde, enthousiasme en aanmoedigingen om mijn talenten te gebruiken, was ik nooit zover gekomen.

Lieve Daan, Lize en Jort, mijn lievelingen. Over een maand denken jullie waarschijnlijk “kan mama niet nog een promotieonderzoek doen” nu ik tijd heb om jullie te herinneren

aan huiswerk maken en kamers opruimen. Dank voor jullie nieuwsgierigheid, humor, zorgzaamheid en geduld.

Lieve Jan, “jij hebt al een boek”, zeg jij altijd als je een boek krijgt. Toch hoop ik dat jij dit boek wél wilt lezen. Zonder jou was dit boek er niet geweest en waren de tabellen/grafieken nooit af gekomen. Door al jouw inspanningen voor dit boekje, verdien je eigenlijk een apart hoofdstuk, toch zal je genoegen moeten nemen met de laatste alinea. Op naar het volgende hoofdstuk in ons leven; bij jou ben ik thuis en kan ik alles aan!

CURRICULUM VITAE

Carolien Renee Meijer was born on August 8, 1983 in Gouda as a part of a dichorial gemelli. After finishing secondary school at the Coornhert Gymnasium in Gouda, she started in 2001 to study Medicine at the Leiden University, the Netherlands. She passed her medical exam in 2007. Carolien began her career in Pediatrics on the ward of het Groene Hart Ziekenuis in Gouda. After 9 months, she started as a pediatrician-in-training at the Leiden University Medical Center (head: Prof. H. Delemarre and supervision of Dr. R. Sukhai) and followed her non-academic part of residency at the Juliana Children's Hospital (head: Dr. F. Brus). During the final phase of her training as a pediatrician, Carolien started to work at the pediatric gastroenterology department under the supervision of Prof. Dr. L. Mearin. After becoming a pediatrician, she worked fulltime at the Lange Land Hospital, Zoetermeer 2016 and the Juliana Children's Hospital Den Haag between 2014-2016. From 2016-2020 she followed a fellowship pediatric gastroenterology (Prof. dr. L. Mearin). During these years, she was principal investigator in several studies, including PreventCD and GLUTENSCREEN. In April 2020 Carolien completed her training as a pediatric gastroenterologist and has been working at the LUMC since that moment.

Carolien is married to Jan Boekel, together they have three children, Daan (2011), Lize (2014) and Jort (2015).