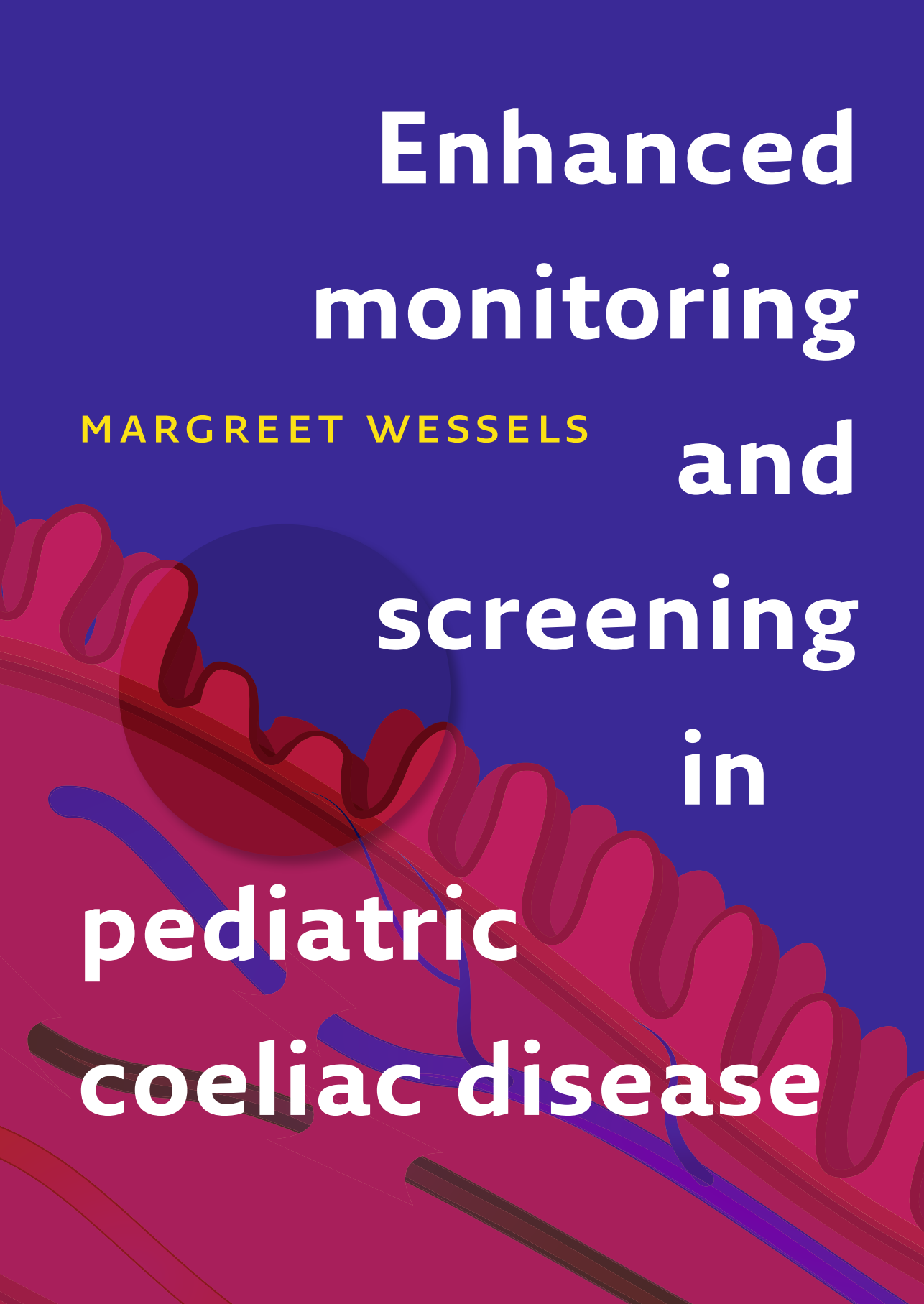
Enhanced monitoring and screening in pediatric coeliac disease

MARGREET WESSELS



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Margaretha Maria Susanna Wessels

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Promotor:

PROF. DR. E.H.H.M. RINGS

Co-promotor:

DR. M.L. MEARIN

Promotiecommissie:

PROF. DR. J.C. ESCHER (ERASMUSMC, LOCATIE SOPHIA KINDERZIEKENHUIS, ROTTERDAM)

PROF. DR. F. KONING

PROF. DR. C.J.J. MULDER (AMSTERDAM UMC, LOCATIE VUMC, AMSTERDAM)

PROF. DR. H. PUTTER

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CHAPTER 1

General introduction and outline of the thesis

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals. It is characterized by anti-tissue transglutaminase type 2 antibodies (TG2A) and enteropathy[1]. In individuals carrying the human leucocyte antigen (HLA)-DQ2 and/or DQ8 haplotype, the ingestion of gluten (a group of proteins present in cereals such as wheat, barley and rye) can cause a T cell-initiated inflammatory response, damaging the small bowel mucosa[2]. In the general population, the prevalence of CD amongst adults as well as children is nearly 1-3%[3,4]. The disease is even more frequent in individuals with other autoimmune diseases such as type 1 diabetes mellitus (T1DM), auto-immune thyroiditis or in specific syndromes such as Down and Turner syndrome (10-15%)[5]. In first degree relatives of coeliac patients, the prevalence is 5-15%[6,7].

Clinical presentation

The disease has a variable clinical presentation, ranging from malabsorption with diarrhoea, abdominal distension and weight loss, to nonspecific signs and symptoms such as fatigue, osteoporosis or iron deficiency anaemia. This diversity of signs and symptoms, in combination with the fact that some individuals do not even have complaints, leads to the so-called ice-berg phenomenon, with most cases of CD being unrecognised and thus untreated[8,9]. These different types of presentation have led to a classification into different subcategories: classical, silent and potential CD (**Table 1**)[10,11]. A high index of suspicion is warranted by doctors but also by the general population, in order to diminish the diagnostic delay most patients encounter[12].

Table 1 *Subcategories of coeliac disease*

Coeliac disease	Symptoms	CD-specific antibodies	Small bowel histological abnormalities
Classical	+	+	+
Silent	-	+	+
Potential	+/-	+	-
Excluded	+/-	-	-

Diagnostic tools

CD-specific antibodies

When CD is suspected, non-invasive tests can be used, measuring CD-specific antibodies (IgA class tissue transglutaminase antibodies, TG2A, anti-endomysium antibodies (EMA) or antibodies against deaminated gliadin peptides (DGPA))[13,14]. Interpretation of these autoantibodies starts with total IgA assessment, since coeliac disease is associated with selective IgA deficiency[15,16]. In IgA deficiency, IgG dependent antibodies can be tested, with IgG EMA, TG2A and DGPA being available. Sensitivity and specificity of both IgA EMA and TG2A and DGPA are high and in concordance with small bowel histological abnormalities: 98% and 90% in severe duodenal lesions, and 97% and 98% in less severe intestinal damage respectively[17].

HLA-genotyping

Furthermore, genotyping for HLA-DQ2 and HLA-DQ8 adds value to the diagnostic scheme, since CD has a strong genetic component. Approximately 90% of coeliac patients carry the HLA-DQ2 haplotype, about 5% the HLA-DQ8 molecule[18–21] and the rest usually one half of the HLA-DQ2 heterodimer (DQA1-0505). The different heterodimers formed by HLA-DQA1* and HLA-DQB1* genes on the surface of antigen presenting cells contribute to the development of CD by the capacity to present gluten to T-cells which initiates the immune response. The HLA-DQ2 and DQ8 haplotypes are present in more than 25% of the general population[20], but only 1% actually develops CD[4]. This indicates that HLA-DQ2 and/or HLA-DQ8 haplotypes are necessary but not sufficient for disease development. In recent years, non-HLA genes have been reported to be associated with CD but with only a modest effect[22,23].

Intestinal histology

Finally, CD is characterized by small bowel mucosa alterations, referred to as gluten-sensitive enteropathy, which is categorized according to the Marsh classification[14]. Marsh classification type 3 or type 2 together with specific coeliac serology support the diagnosis of CD. These small bowel biopsies are obtained by means of esophago-gastro-duodenal endoscopy, an invasive method, especially in children needing general anaesthesia or deep sedation for the procedure. Until recent years, the histological assessment of duodenal biopsies has been the gold standard for the diagnosis of CD. However, patchiness of villous atrophy[24,25] but also difficulties with regard to proper interpretation, cutting and orientation of duodenal biopsies in order to come to a precise Marsh-classification can lead to false negative but also false positive biopsy results. Therefore, the histological interpretation needs to be done by an experienced pathologist with the patient's clinical complaints, serology and HLA-type in mind. In 2012, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) proposed an exception with regard to performing duodenal biopsies for a well-defined

group of children, having high titers of TG2A (>10 times upper limit of normal), positive EMA and positive HLA-DQ2/DQ8[1]. This strategy has been shown to be valid in 2017 by a multicentre European prospective study[26]. However, it still raises a lot of debate in adults suspected of CD and it is challenged for example by pediatric gastroenterologists from the United States of America[27].

Treatment

CD can be successfully treated with a gluten free diet (GFD) which restores small bowel histology and improves symptoms. However, this diet may be difficult to follow and may lead to social constraints. It is known that dietary adherence differs among individuals, with noncompliance varying from 25-50% among children and adolescents[28-30]. 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 transport. Contamination can also take place during food-processing either in factories or at home. The capacity of gluten to improve the properties of food and non-food such as pencils and glue, increases the risk of contamination even more. Besides the impact of a GDF on a patient's daily and social life, the GFD can also lead to nutritional deficiencies since gluten-containing cereals like wheat, barley and rye are important sources of dietary iron, calcium, folate and vitamin B12[28,31,32,33]. Gluten-free grains such as buckwheat or quinoa are naturally rich in group B vitamins[34] but commercially available gluten-free products do not contain the same amount of iron, vitamin B12 and folate as the wheat flour products that they aim to replace[35,36]. Therefore, testing for anaemia, iron status and calcium, folic acid, vitamin B12 and vitamin D is common practice in patients with CD treated with a GFD. At the time of initiating this thesis, studies investigating the actual presence of nutritional deficiencies in children on a GFD, however, were lacking.

Follow-up

Despite the knowledge that non-compliance often occurs, a gold standard to assess compliance is lacking[37]. An extensive dietary evaluation by a trained dietitian is considered the best method[38], but not very practical due to its time-consuming nature. Repeat duodenal biopsies to monitor mucosal recovery is usually not a practical option, especially in children wherein endoscopy to obtain biopsies is done under anaesthesia or deep sedation. Serologic testing is not sensitive enough to detect infrequent gluten exposure[39-41], although it is usually performed in CD patients who are on a GFD. When this thesis commenced, several short dietary questionnaires had been developed in order to save time and to address compliance in a standard manner. However, they were tested only in adult CD patients and not in the pediatric CD population.

Risk groups

The Dutch and European CD guidelines recommend testing for CD in asymptomatic individuals with increased risk for CD: other autoimmune diseases such as T1DM, autoimmune thyroid and liver disease, Down, Turner and Williams syndrome, selective IgA deficiency and first degree relatives (FDRs) of coeliac patients[1,42]. Because of the high negative predictive value of HLA-typing for CD, unnecessary investigations in HLA-DQ2 and DQ8 negative individuals can be avoided. Therefore, HLA-typing can be offered to these individuals, albeit that due to its high costs in combination with a shared genetic background already predisposing to the same HLA-type, it is debated in certain risk groups, such as T1DM[43,44]. At the time of initiating this thesis, the impact of screening for CD on parents and perceived health of FDRs had not been studied, neither was the best suited screening frequency.

With regard to children with diabetes, several consensus based guidelines have different screening and treatment recommendations. Some suggest to screen all T1DM patients for CD[1,45,46], but state that while it seems sensible to put also an asymptomatic child on a GFD to avoid the development of complications, limited data are available to support this. Conversely, other guidelines advise screening only in symptomatic T1DM patients and emphasize informing parents that the treatment of asymptomatic CD in T1D is controversial[47,48]. Despite high sensitivity and specificity, the interpretation of TG2A and EMA in children with T1DM has proven difficult. The 2012 ESPGHAN guideline recommends duodenal biopsies if TG2A titers are >3xULN in asymptomatic individuals[1]. However, elevated TG2A titers often show spontaneous normalization in children with T1DM[49] and people at genetic risk for CD (like children with T1DM) have more often false-positive TG2A results[50]. Altogether, it leaves clinicians without a concrete method of patient management and speak to the absence of available literature for development of an evidence-based approach.

Outline of this thesis

With much attention in CD related research on diagnostics, prevention and new therapeutic modalities, the focus of this thesis has been on two clinical aspects of pediatric CD: the monitoring methods used during follow-up (Part I) and the screening process of individuals at risk (Part II). The specific questions addressed in my thesis are presented in **Table 2**.

Table 2 *Questions addressed in this thesis*

1	Do nutritional deficiencies persist in coeliac children after start of a GFD?
2	Do short GFD questionnaires detect infrequent dietary transgressions in coeliac children?
3	What is the impact of HLA-screening in children at risk for coeliac development?
4	What is the best screening method in FDRs of newly diagnosed coeliac patients?
5	When should duodenal biopsies be performed in T1DM children with elevated TG2A serology, since serology is often found to be false positive and/or declining spontaneously in these children?

Part I Follow-up

In **Chapter 2**, the results of a retrospective analysis of all complementary serologic investigations done at time of diagnose and during annual follow-up of children with CD are presented in order to describe the course of nutritional deficiencies after treatment. **Chapter 3** investigates whether a short standardized dietary questionnaire correlates with the dietary interview performed by a dietician in children with CD and how both match with CD specific serology.

Part II Risk Groups

The impact of HLA-typing in healthy children from coeliac families on parents is discussed in **Chapter 4**, together with the parental knowledge on the genetic background of CD and whether they would repeat HLA-typing in future children. **Chapter 5** also addresses coeliac families and describes the effect of sex, HLA-type and age of FDRs at time of CD diagnosis in the index coeliac patient in order to establish a better screening protocol for these high risk individuals. **Chapter 6** challenges the current recommendation for asymptomatic diabetic children with TG2A titers >3x the upper limit of normal (ULN) to be biopsied by means of Receiver Operating Characteristic (ROC) analyses of TG2A levels and corresponding duodenal histology.

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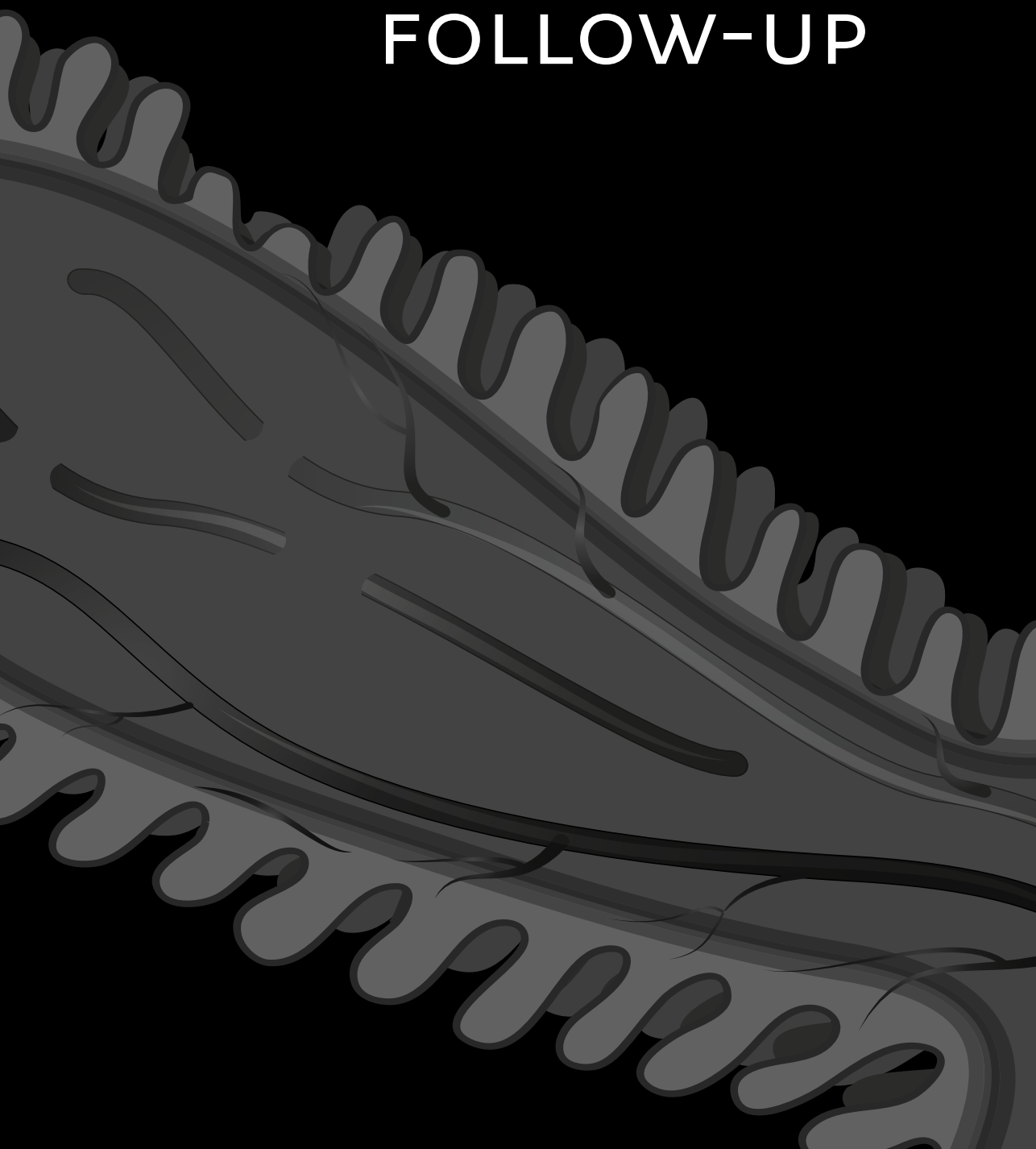
PART I

FOLLOW-UP



CHAPTER 2

Complementary serologic investigations in children with coeliac disease is unnecessary during follow-up

J PEDIATR. 2016 FEB;169:55-60

Wessels MMS

van Veen II

Vriezinga SL

Putter H

Rings EHHM

Mearin ML

Abstract

OBJECTIVES To determine the frequency of nutritional deficiencies and thyroid dysfunction in children with coeliac disease (CD) at diagnosis and during follow-up after initiation of a gluten-free diet, since laboratory investigations of hemoglobin, ferritin, calcium, folate, vitamin B12, vitamin D and thyroid function are regularly ordered in CD children despite sufficient evidence for these.

METHODS Between 2009 and 2014, test results of hemoglobin, ferritin, folate, vitamin B12, calcium, vitamin D-25-OH, FT4 and TSH of CD children regularly seen at the Leiden University Medical Center were investigated. Laboratory reference ranges were used to define abnormal results. Pearson's chi-square test for trend, unpaired t-test and one-way ANOVA were used for statistical analysis.

RESULTS 182 children were evaluated, wherein 119 were new diagnoses. On average, 17% of results per year were missing due to incomplete blood investigations. Iron deficiency (28%) and iron deficiency anemia (9%) were found upon CD diagnosis. Folate (14%), vitamin B12 (1%) and vitamin D deficiencies (27%) were also seen. No hypocalcemia or thyroid dysfunction was found. At follow-up, iron deficiency, iron deficiency anemia, folate and vitamin D deficiency were respectively observed in 8%, 2%, 3% and 25% of patients. No vitamin B12 deficiency, hypocalcemia or thyroid disease was found.

CONCLUSION Complementary blood investigations are relevant at time of CD diagnosis but have little diagnostic yield during follow-up visits once the patient is placed on a gluten-free diet. Thus, we recommend that these variables only be assessed on indication, such as fatigue or abnormal growth.

Introduction

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals. It is characterized by anti-tissue transglutaminase type 2 antibodies (TG2A) and enteropathy[1]. The disease can be successfully treated with a gluten-free diet (GFD)[2]. Small bowel mucosal damage in CD patients can lead to malabsorption and, subsequently, nutritional deficiencies causing osteoporosis, iron deficiency (ID) or iron deficiency anemia (IDA). Since gluten-containing cereals like wheat, barley and rye are important sources of dietary iron, calcium, folate and vitamin B12, the treatment of CD with a GFD can also lead to nutritional deficiencies[3-6]. Gluten-free grains such as buckwheat or quinoa are naturally rich in group B vitamins[7] but commercially available gluten-free products do not contain the same amount of iron, vitamin B12 and folate as the wheat flour products that they aim to replace[8,9]. A lack of variation in food choices, often seen in CD children[10], may aggravate the problem[11]. It is common practice to check the CD patients' ID/IDA indices (i.e. a complete blood count, including mean corpuscular volume, red cell distribution width, serum ferritin), calcium, folate and vitamin B12 levels, both at diagnosis and at follow-up. However, there is limited information on the incidence of nutritional deficiencies in patients with treated CD. Some evidence based CD guidelines such as the one from the National Institutes of Health (NIH)[12] and the Dutch Society for Gastroenterology[13] recommend that all aforementioned blood tests continue to be performed in patients who already receive ongoing medical treatment for their CD. Other CD guidelines such as those by the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)[1], the National Institute for Health and Care Excellence (NICE)[14] or the North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN)[15] provide no guidance on the matter. In addition, several guidelines recommend testing for thyroid autoimmunity at various intervals but give no information on how frequently this should be done[13,16].

Our study's primary aims were to assess the frequency of nutritional deficiencies, specifically iron (and the iron deficiency anemia that may follow), calcium, folate and vitamin B12, and to determine the presence of thyroid dysfunction among CD children at the time of diagnosis and at follow-up while on a GFD. The secondary aim was to determine whether these investigations were necessary in the routine follow-up of children with treated CD.

Methods

We analyzed the blood testing results of all CD children who had medical checks between January 2009 and January 2015 at the Leiden University Medical Center (LUMC).

CD was diagnosed according to the ESPGHAN criteria[1]. After diagnosis, these children were then seen regularly according to (inter-)national guidelines. These visits included blood investigations[1,13], particularly CD-specific antibodies, hemoglobin (determined by Sysmex XE-2100), ferritin, folate, vitamin B12 (all measured by ECLIA using Roche Modular E170), free thyroxin (FT4) and thyroid stimulating hormone (TSH) (both determined by colorimetric assay IFCC). Calcium levels (measured by Roche Modular P800) and vitamin D-25-OH (determined by ECLIA using Roche Modular E170) were only recorded beginning in 2012 because our department had only started doing these routine investigations in CD patients after 2011. Laboratory reference ranges per blood parameter are shown in **Table 1**. IDA was defined as ID plus anemia[17]. Hypothyroidism was defined as an FT4 < 10 pmol/L and TSH > 4.8 mU/L while hyperthyroidism was defined following an FT4 > 24 pmol/L and TSH < 0.3 mU/L.

Table 1 *Laboratory reference range used to define abnormal results.*

Biochemical parameter	Limit of abnormal value
Hemoglobin, age <7 years	< 6.9 mmol/L (< 11.0 g/dL)
Hemoglobin, age 7-15 years	< 6.5 mmol/L (< 10.4 g/dL)
Hemoglobin, age >15 years	< 6 mmol/L (<9.6 g/dL)
Ferritin, age <5 years	< 12 ug/L
Ferritin, age ≥5 years	< 15 ug/L
Folate	< 10 nmol/L (< 4.45 ng/mL)
Vitamin B12	< 150 pmol/L (203 pg/mL)
Calcium	< 2.15 mmol/L
Vitamin D-25-OH	< 50 nmol/L (< 20.8 ng/mL)
Thyroid Stimulating Hormone	< 0.3 mU/L > 4.8 mU/L
Free Thyroxin	< 10 pmol/L (< 0.78 ng/dL) > 24 pmol/L (< 1.86 ng/dL

We registered the following patient data: sex, date of birth, age at CD diagnosis, coeliac antibodies, HLA-typing, Marsh histologic classifications of the diagnostic small bowel biopsies, and date of blood extraction. The time of CD diagnosis was defined as the date of diagnostic small bowel biopsies or, if there was no indication for a diagnostic biopsy, the date when high titers of endomysial antibodies (EMA) and TG2A in the serum were

confirmed[1]. Furthermore, we recorded the presence of hypo- or hyperthyroidism at the time of diagnosis or its subsequent development during follow up. Prescribed supplementation therapy for hypothyroidism and deficiencies was also noted.

Laboratory investigations performed from 6 months prior to and 3 months after the diagnosis were considered as blood tests "at time of diagnosis". The first year follow up blood tests were taken between 9 and 18 months post-diagnosis while the second year follow up tests were done within 1.5 to 2.5 years of CD diagnosis, the third year follow up between 2.5 and 3.5 years from diagnosis, and so on. If multiple samples for one parameter were available at one time period, the most abnormal result was used for analysis. If laboratory results were unavailable, they were recorded as missing values. Blood samples taken more than 5.5 years after diagnosis were not used for analysis. Blood tests done after supplementation of iron or vitamins in order to evaluate treatment effects were not considered in the analysis.

Data analysis

Where appropriate, Pearson's chi-square test for trend, unpaired t-test and one-way ANOVA were used. A two-tailed probability of $p < 0.05$ was considered significant. Statistical analysis was performed using Statistical Package for the Social Sciences (IBM, version 20; SPSS Inc., Chicago, IL, USA). No approval from a Medical Ethical Committee was needed for this study since the blood tests were standard of care and analysis was done anonymously.

Results

Patient characteristics are shown in **Table 2**. There were a total of 182 children evaluated, wherein 119 children were newly diagnosed during the study period. The other children were diagnosed prior to 2009 or only had follow-up investigations because CD was previously diagnosed in another hospital. The distribution of age, age at CD diagnosis, mean follow-up duration, Marsh classification and HLA-typing were similar in girls and in boys (data not shown).

Laboratory results

In all participants, 436 blood investigations were performed: 119 times at the time of CD diagnosis and 317 times during follow-up visits. On average, 17% of the children had incomplete annual blood investigations, where it can further be observed that 58% of this group did not have vitamin D tests done.

Table 2 *Characteristics of 182 children with coeliac disease (CD) having medical checks between January 2009 and December 2014.*

Sex, % female	65
Ethnicity, %	
European	93
(North) African and Turkish	4
Asian	2
Unknown	1
Age at diagnosis, mean in years (SD)	6.3 (± 4.3)
Duration of follow-up, mean in years (SD)	3.1 (± 3.1)
Diagnosis without biopsies (ESPGHAN criteria), nr	28
Biopsies confirmed CD, nr	154
Histology small bowel biopsies at diagnosis, %	
Biopsies performed in another center without report available	1 4^
Marsh 2	25
Marsh 3a	49
Marsh 3b	21
Marsh 3c	
HLA-typing result, %	
DQ2 or DQ8 positive	94
Unknown	6
IgA level, %	
>0.2 g/l	96
<0.2 g/l	4
CD specific antibodies at diagnosis, %	
EMA and/or TG2A positive	97
EMA and TG2A negative*	1
EMA and TG2A unknown†	2

^ All with high levels of anti-endomysial antibodies (EMA) and/or anti-tissue transglutaminase type 2 antibodies (TG2A).

* Diagnosis at age 16 months presenting with malabsorption and failure to thrive, small bowel biopsies Marsh 3a and (very) good response to a gluten-free diet.

† CD diagnosed in another hospital, all Marsh 3 proven at biopsy.

At diagnosis

The results of the laboratory tests are shown in **Table 3**. The mean hemoglobin value in the children with ID and IDA was 6.6 mmol/L (SD 0.2). The ten children with IDA were

significantly younger than the others (mean 2.64 years SD 1.1; 6.5 years SD 4.3 respectively, p < 0.001). All children showed normalization of their hemoglobin without any prescribed iron supplementation a year after a GFD, except for a 3 year old girl whose hemoglobin level remained low (6.7 mmol/L) despite supplementation. The mean folate level in children with folate deficiency was 7.7 nmol/L (SD 1.4). The age at diagnosis was similar among the children with and without folate deficiency (mean age 7.6 years SD 6.4; 6.2 years SD 4.1 respectively, p = 0.23). Normalization of folate occurred within one year after starting the GFD in all folate-deficient children regardless of supplementation status. Of note, 40% of the children with folate deficiency were prescribed supplements. One child with vitamin B12 deficiency (64 pmol/L) and abnormal homocysteine and

Table 3 *Frequency of deficiencies and thyroid dysfunction in children with coeliac disease at the time of diagnosis and during follow-up.*

Variable assessed between January 2009 and December 2014	**Diagnosis n=119* (%)**	**1st Year n=83* (%)**	**2nd Year n=79* (%)**	**3rd Year n=57* (%)**	**4th Year n=50* (%)**	**5th Year n=48* (%)**
Iron deficiency#	29/104 (28)	4/79 (5)	4/77 (5)	4/57 (7)	4/48 (8)	2/48 (4)
Iron deficiency anemia##	10/110 (9)	2/81 (2)	1/78 (1)	1/57 (2)	0/49	0/47
Folate deficiency^	12/84 (14)	0/73	2/71 (3)	0/55	0/40	0/44
Vitamin B12 deficiency^^	1/85 (1)	1**/73 (1)	1**/71 (1)	0/55	0/40	0/44
Elevated Thyroid Stimulating Hormone (TSH)‡	12/99 (12)	10/76 (13)	7/71 (10)	3/55 (5)	3/46 (7)	9/47 (19)
Hypo‡ ‡/hyperthyroidism‡ ‡ ‡	0/99	0/79	0/73	0/54	0/46	0/47
Variable assessed between January 2012 and December 2014	**Diagnosis n=71* (%)**	**1st Year n=50* (%)**	**2nd Year n=43* (%)**	**3rd Year n=36* (%)**	**4th Year n=26* (%)**	**5th Year n=31* (%)**
Hypocalcemia±	0/65	0/37	0/34	0/25	0/14	0/31
Vitamin D deficiency±±	8/30 (27)	9/48 (19)	7/42 (17)	4/34 (12)	3/22 (14)	7/28 (25)

* Total number of children at different time points.

Ferritin < 12 µg/L in children < 5 years of age or Ferritin < 15 µg/L in older children; ## Iron deficiency plus anemia (Hemoglobin < 6.9 mmol/L if age < 7 years, < 6.5 mmol/L if age 7-15 years, < 6.0 mmol/L older children); ^ Folate < 10 nmol/L; ^^ Vitamin B12 < 150 pmol/L; ‡ TSH > 4.8 mU/L; ‡ ‡ Free Thyroxin 4 < 10 pmol/L and TSH > 4.8 mU/L; ‡ ‡ ‡ Free Thyroxin 4 > 24 pmol/L and TSH < 0.3 mU/L.

** 1 girl with normal homocysteine and methylmalonic acid ruling out true vitamin B12 deficiency. ± Calcium < 2.15 mmol/L; ±± Vitamin D-25-OH < 50 nmol/L.

methylmalonic acid levels had folate deficiency as well (these tests were performed in a referring hospital, thus, the exact data could not be retrieved). Both folate and vitamin B12 had normalized six months after their respective supplementations.

Anthropometric evaluation of the children with iron, folate and vitamin B12 deficiencies, done in order to see whether these relatively young children had a classic presentation of CD with malabsorption, showed stunting (defined as height < -2.0 SDS) and underweight (defined as weight for height < -1/5 SDS) in 30% and 15% of the children. In all children, recovery of height and weight was seen while on a GFD.

The mean level of vitamin D-25-OH in deficient children was 38 nmol/L (SD 6.8). Only 25% of these children were prescribed with vitamin D supplements (i.e. calcium carbonate/ vitamin D 500 mg/400 IU, for 3-6 months), yet normalization of values occurred in all of these children after one year, except for two adolescents who did not receive these prescriptions. The mean age of the children with vitamin D deficiency at diagnosis was significantly higher compared to the children with normal vitamin D levels (mean 7.6 ± SD 4.6 and mean 5.9 ± SD 4.1 respectively, p=0.03). No child had hyper- or hypothyroidism. Prior to 2009, Graves' disease and Hashimoto's thyroiditis were diagnosed in 1 and 3 patients respectively, both prior to the development of CD. The male-female ratio was similar among the children with and without thyroid deficiencies, and with and without elevated TSH levels (data not shown).

During follow up

The results of the laboratory tests are shown in **Tables 3 and 4**. In the first 3 years after diagnosis, 3 girls developed IDA (mean hemoglobin 6.6 mmol/L SD 0.2) and 1 girl had persistent IDA (hemoglobin 6.7 mmol/L; She had existing IDA at time of CD diagnosis and it continued on despite prescribed iron supplementation post-diagnosis). These girls were significantly younger compared to the children without IDA (mean 3.4 years SD 1.4, and 6.5 years SD 4.3, at time of IDA respectively, p = 0.02).The hemoglobin and ferritin levels normalized in the rest of the girls within 1 year of CD diagnosis. Iron supplementation was only given to one of these patients.

Two patients (3%) developed mild folate deficiency (folate levels 8.7 and 9.5 nmol/L) during the second follow-up year. Supplementation was given to one of them, a 12-year old boy, with normalization of folate after 6 months. Supplementation was withheld in the other patient, a 17 year old asymptomatic girl, since her folate level was only marginally low (9.5 nmol/L). Her follow-up measurements could no longer be obtained after she transferred to the gastroenterology department of another hospital. One adolescent had low vitamin B12 at the first and second year visits, but since normal levels of homocysteine and methylmalonic acid were found, true vitamin B12 deficiency was ruled out and no supplementation was thus prescribed.

Table 4 *Summary of the literature on the prevalence of iron, vitamin B12 and folate deficiency in coeliac disease patients*.*

Study and year published	Study population	No. of patients	Nutrient deficiency** at diagnosis	Nutrient deficiency during follow-up
Bonamico M[39] 1987	Children	80	Iron deficiency (56%)	Not available
Dahele A[40] 2001	Adults	39	Iron deficiency (49%) Vitamin B12 deficiency (41%)	Vitamin B12 deficiency resolved after one year gluten-free diet
Kemppainen T[41] 1998	Adults	40	Folate deficiency (35%) Iron deficiency (32.5%)	Folate and iron deficiency 8% and 22.5% after one year gluten-free diet respectively
Dickey W[42] 2002	Adults	159	Vitamin B12 deficiency (12%)	Not available
Haapalahti M[18] 2005	Adolescents and young adults	26	Iron deficiency (28%) Folate deficiency (31%) Vitamin B12 deficiency (12%)	Not available
Bergamaschi G[19] 2008	Adults	132	Iron deficiency (34%)	30% "*some degree*" of iron deficiency after one year with GFD
Fernandez A[43] 2010	Adults	68	Iron deficiency (49%) Folate deficiency (24%)	Not available
Botero-Lopez JE[20] 2011	Children and adults	73	Iron deficiency (45%)	Not available
Wierdsma NJ[21] 2013	Adults	80	Iron deficiency (46%) Folate deficiency (20%) Vitamin B12 deficiency (19%)	Not available
Gokce S[44] 2014	Children	191	Iron deficiency (8%)	Not available

* By means of Medline search from 1980 until December 2014 using coeliac disease, anemia, iron deficiency, folate deficiency, vitamin B12 deficiency, nutritional deficiencies and nutritional status as Mesh terms.

** Defined as levels of hemoglobin, ferritin, folate and vitamin B12 below reference values.

Vitamin D deficiency (mean vitamin D-25-OH 38.5 nmol/L, SD 7.7) was present in up to 25% of the patients. Calcium carbonate/ vitamin D, once daily 500 mg/400 IE, was prescribed in 40% of deficient children, with the levels returning to normal in a third of these children.

No hyper- or hypothyroidism was found during our follow-up period. However, a 10 year old asymptomatic girl, whose mother was known to have hypothyroidism due to a rare TSHR-gene mutation (C.1631G>A), was diagnosed with subclinical hypothyroidism secondary to the same genetic defect (FT4 11.8 pmol/L; TSH 12.8 mU/L). Elevated TSH levels (mean 6.2, range 4.8-13.6, SD 1.6) were seen in 33 patients. It was noted to occur once

in 48% of them and repeatedly in 39% . The high TSH values only normalized in 17% of these patients. However, all children with repeatedly elevated TSH levels had negative thyroperoxidase antibodies (AbTPO). Two patients developed hypothyroidism after the 5th year of CD follow-up. Both children complained of fatigue and showed decelerating growth. By accounting for thyroid dysfunction prior to CD diagnosis and beyond our follow-up period (after 5.5 years of follow-up), the prevalence of hypothyroidism in our cohort was 3.2% (n=6, 4 female) and hyperthyroidism, 0.5% (n=1, male).

Discussion

As far as we know, this is the first study on the outcome and relevance of complementary blood investigations in the follow-up of children with CD. The results indicate that these investigations are relevant at the time of diagnosis because up to 28% of the children presented then with varying iron, folate and vitamin B12 deficiencies. However, ordering these tests at patient follow-up visits may be questionable since only mild deficiencies occurred in a minority of the children (5-10%). This outcome has implications in the organization of care for CD children because blood tests are time-consuming and expensive. As of 2014, this costs approximately €150-200 per patient, merely for extracting and handling blood samples in our laboratory, and exclusive of coeliac serology charges.

There is limited information on the incidence of nutritional deficiencies in patients with treated CD. Published data vary widely, most probably because they have evaluated small and heterogeneous patient groups focusing on certain nutritional deficiencies, only at time of diagnosis (**Table 4**). In general, the nutritional deficiencies at diagnosis found in our study were noted to occur similarly or even less frequently than earlier studies, except for vitamin B12 deficiency. This deficiency was seen to be much lower in our cohort (2% versus 12-41% found in adolescents and adults)[18-21]. The absence of IDA, folate and vitamin B12 deficiency during follow-up may be explained in two ways. First, adherence to the GFD leads to recovery of the intestinal mucosa, thereby normalizing nutrient absorption. Second, dietary counselling is offered to the patients after diagnosis. This includes daily nutritional intake of iron, folate and vitamin B12[5]. Interestingly, a recent study in adult CD patients showed an increased use of over the counter supplements simultaneous with a GFD treatment[22], something that we did not investigate in our cohort. In the Netherlands, over the counter use of supplements in children is uncommon. Dietitians, whose role is to provide advice on GFDs, likewise do not promote its use. However, the fact that we only recorded prescribed supplements may have underestimated the prevalence of deficiencies at follow-up.

ID and IDA were infrequently seen during follow-up visits. These values were considerably less compared to the rest of the children from the general Dutch population aged 6-36 months. In the latter group, the frequency of ID was 18.8% and IDA, 8.5%[23]. Moreover, the frequency of ID in our patients is lower than the reported 17% among healthy Finnish adolescents[18]. Therefore, it may be questioned whether these deficiencies are related to CD or merely reflect its presence in the general child population .

Our findings on the frequency of thyroid dysfunction (3.7%) are similar to the ones from previous studies, with the prevalence of thyroid autoimmunity (elevated TSH or presence of AbTPO), hypothyroidism and hyperthyroidism varying from 10-26%[24,25], 2-6%[24,25] and 1%, respectively[24,25]. The rationale behind thyroid function testing as part of a CD patient's follow up rests on the fact that there exists a high frequency of thyroid autoimmunity in CD[24,26]. In addition, there is conflicting evidence on the GFD's protective effect in the development of auto-immune thyroid disease[27,29]. However, the clinical relevance of elevated TSH is debatable since elevated TSH levels can fluctuate or normalize, as was seen in our patients. They were also observed to occur or persist, in the absence of AbTPO and without the development of clinical hypothyroidism. Furthermore, thyroid disease was only diagnosed in symptomatic children whose family history and clinical presentation were suggestive of hypo-/ hyperthyroidism.

The strength of our study is the relatively large patient group with well-documented CD, most likely representative of the West European pediatric CD population. The long follow-up period allowed us to demonstrate the natural course of nutritional deficiencies after treatment with a GFD.

One limitation of our study is an incomplete annual laboratory measurement, despite its availability in the majority of cases. Most missing laboratory investigations occurred due to insufficient blood obtained at venipuncture. We believe that since the analysis of calcium and vitamin D took place in a large group of patients within a short follow-up period, the values obtained represent the general population of coeliac children.

One could argue that deficiencies during follow-up might reflect non-compliance to the GFD and therefore, a degree of malabsorption. We have thus retrospectively examined the TG2A levels in children with IDA and folate deficiency and found them all to be normal, thus confirming the patient compliance.

It is known that CD can lead to a diminished bone mineral density in 40-66% of the coeliac patients at diagnosis[30,31], with low calcium levels in 18-24%[31,32]. In coeliac children and adults, a GFD has proven to be effective in ameliorating bone mineralization[31,33]. Vitamin D deficiency has been found to equally occur at diagnosis and during follow-up[32,34]; this

is similar to our results. It is known that vitamin D deficiency occurs in up to 20-70% of children, regardless of age, sex, socio-economic status and dietary supplementation. The main variation in its occurrence may easily be explained by race or ethnicity and seasonal influences, i.e. it is more commonly observed among darker individuals owing to differing skin pigmentation and in the winter due to reduced sun exposure[35-38]. Our analysis indicates that vitamin D status depends on more than a gluten-free diet and supplementation, considering that 2/3 of patients who were prescribed supplements to correct vitamin D deficiency still did not achieve normal levels. Therefore, it seems that vitamin D deficiency may not be directly linked to CD, but merely represents its frequency in the general population. However, assessment of vitamin D status and correcting the deficiency or ensuring its spontaneous resolution can be generally considered as good patient care because of the known effects of untreated CD on bone health.

Conclusion

We have shown that at the time of pediatric CD diagnosis, iron deficiency, iron deficiency anemia, and folate deficiency occur frequently. However, the vast majority of these values normalize after a GFD treatment, even without the prescribed supplementations. The low frequency of deficiencies at follow-up may not even be related to CD, since they are also found to the same degree in the general pediatric population. Furthermore, we have shown that vitamin B12 deficiency only sporadically occurred at the time of CD diagnosis, whereas hypocalcemia did not occur at all. Presence of thyroid disease in our cohort was low and occurred only in symptomatic children. We therefore recommend that these variables only be evaluated on indication in follow up CD visits, for example, after specific complaints such as fatigue or abnormal growth.

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CHAPTER 3

Assessment of dietary compliance in coeliac children using a standardized dietary interview

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Wessels MMS

te Lintelo M

Vriezinga SL

Putter H

Hopman EG

Mearin ML

Abstract

BACKGROUND & AIMS Compliance to a gluten-free diet (GFD) in coeliac disease (CD) is ideally assessed by dietary interviews, albeit time-consuming. Short dietary questionnaires have been developed for adults but not for children. Primary aim was to compare GFD compliance in coeliac children, measured by a short dietary questionnaire against a dietary interview. Secondary aims were correlation between both questionnaires and coeliac antibodies and identifying variables predicting noncompliance.

METHODS Between 2012 and 2014, participants in the eHealth CoelKids study, completed a short dietary questionnaire and standardized dietary interview together with measurement of anti-tissue transglutaminase antibodies (TG2A). Results of the questionnaires were assigned under similar categories. Factors possibly influencing dietary compliance were recorded. Where appropriate, Pearson's Chi-square test for trend, unpaired t-test, Cohen's kappa and one-way ANOVA were used.

RESULTS 151 of 165 participating patients were studied, 66% were female. Mean age was 11.3 years (2-26, SD 5.4), mean age at CD diagnosis was 4.9 years (1-23, SD 4.0). The short questionnaire and dietary interview correlated poorly, detecting problems in dietary adherence in 14% and 52% of the patients, respectively (Cohen's kappa 0.034). Only the short questionnaire correlated with TG2A ($p = 0.003$). Only older age was associated with noncompliance, the mean age of completely nonadherent, adherent but committing errors, and strictly adherent patients were 15.5, 11.5 and 10.1 years, respectively ($p<0.001$).

CONCLUSION Compared to the dietary interview, short dietary questionnaires and TG2A serology failed to detect dietary transgressions in CD children, wherein adolescents were shown to be at highest risk.

Introduction

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten in genetically susceptible individuals and is characterized by anti-tissue transglutaminase type 2 antibodies (TG2A) and enteropathy[1]. In individuals carrying the HLA-DQ2 and/or DQ8 haplotype, the ingestion of gluten (a group of proteins present in cereals such as wheat, barley and rye, can lead to a T cell-initiated inflammatory response, damaging the small bowel mucosa[2]. CD is a common disorder, occurring in approximately 1-3% of the general population[3,4].The disease has a variable clinical presentation, ranging from malabsorption with diarrhea, abdominal distention and weight loss, to nonspecific signs and symptoms such as fatigue, osteoporosis or iron deficiency anemia. CD can be diagnosed by the detection of CD-specific antibodies (usually IgA class tissue transglutaminase antibodies TG2A and anti-endomysium antibodies)[5,6] and small bowel biopsies that show characteristic histological alterations. CD can be successfully treated in most cases with a gluten-free diet (GFD) which restores small bowel histology and improves symptoms. However, this diet may be difficult to follow and may lead to social constraints. It is known that dietary adherence differs among individuals, with noncompliance varying from 25-50%[7-9] among children and adolescents. Despite the absence of a gold standard to assess dietary compliance[10], a dietary evaluation by a trained dietitian is considered the best method[11]. Repeat duodenal biopsies to monitor mucosal recovery is usually not a practical option, especially in children wherein endoscopy to obtain biopsies is done under anesthesia or deep sedation. Serologic testing is not sensitive enough to detect infrequent gluten exposure[12-14]. It has been shown that adults tend to overestimate their level of compliance if they are asked to self-report it[15]. Furthermore, information about the trustworthiness of adherence as reported by parents show a broad range[16]. Food diaries and questionnaires are frequently used in CD research in order to estimated gluten intake. These are, however, mostly used in order to assess the diet's nutritional content[17,18] and have not been validated, except for food questionnaires in infants[19] and children aged 1-4 years[20]. A dietary interview to assess compliance is time-consuming, taking 20-30 minutes per patient, and requires expert personnel. Several short questionnaires have been developed to measure GFD adherence in order to save time and address compliance in a standardized manner. For example, a questionnaire developed in Italy and tested in coeliac adults[13] consists of four questions that take less than a minute to administer. Moreover, it may be filled out even by non-expert personnel or by patients themselves online. To our knowledge, these short questionnaires have never been tested in children, adolescents or young adults.

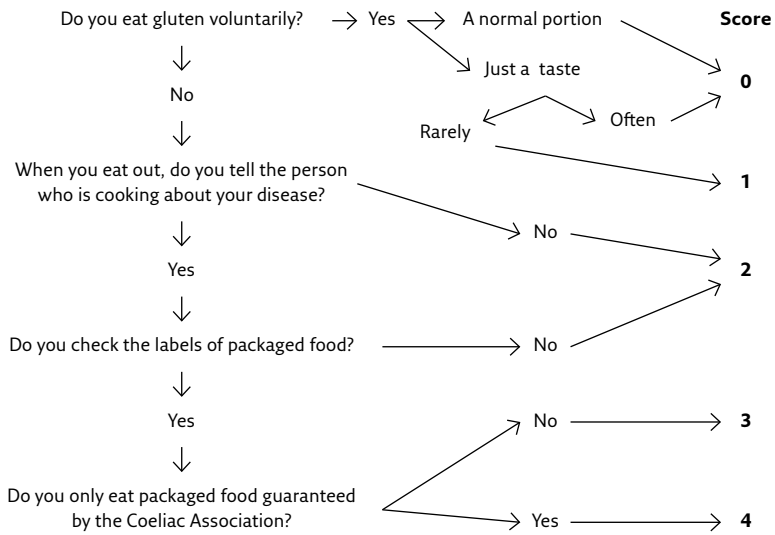
The primary aim of this study was to compare GFD compliance in CD children, adolescents and young adults as measured by a short and standardized dietary questionnaire that has been validated in coeliac adults[13] against a standardized dietary interview.

Secondary aims include 1. the assessment of the correlation between the short dietary questionnaire, standardized dietary interview and coeliac-specific serum antibodies, and, 2. the identification of risk factors for noncompliance with the gluten-free diet.

Materials and methods

For this cross-sectional study, dietary compliance was assessed in coeliac children and young adults participating in the eHealth CoelKids study and who were randomized to receive the usual health care (www.coelkids.nl). Patients were recruited between May 2012 and July 2014. They became eligible if CD was diagnosed according to ESPGHAN criteria[1,21] at least 1 year before enrolment and patients (or their parents) could complete the online questionnaires in Dutch. As part of the CoelKids project, the patients and/or parents were asked to complete two questionnaires about dietary compliance. The first questionnaire consisted of a Dutch adaptation of a short questionnaire validated in Italian CD adults to assess GFD compliance, hereafter referred to as the "short questionnaire"[13] (**Figure 1**) in this article.

Figure 1 *Short diet questionnaire validated in coeliac adults[13]*



'Often': the patient consumes gluten quite frequently that he/she cannot remember when and how many times that has happened. 'Rarely': the patient consumes gluten only occasionally. She/he can remember when and how many times that has happened.

The scores obtained from this questionnaire were divided into three scores: 1 — indicating GFD not followed, 2 — GFD with important errors, 3 — strict GFD. The second questionnaire, hereafter referred to as the "dietary interview questionnaire", consisted of an extensively written patient interview developed by one of the authors (EH) who is an experienced dietitian specializing in CD and GFD. This 26-item questionnaire reflects the patient interview that was verbally conducted during regular face-to-face consultations with the dietitian to evaluate the GFD (**Supplemental material appendix C**). This interview was standardized and converted into a written version for this project. It addresses several domains, including GFD compliance and patient (or parent) knowledge and attitude toward the GFD. For example, they were asked about gluten-free food preparation, the reading of food labels, and the need for extra information/ contact with a dietitian or medical doctor. For optimal comparison, the final scores of the dietary interview questionnaire were grouped into the same three scores obtained from the short questionnaire. In addition, to improve user-friendliness, a comprehensive 11-item version of the dietary interview was also tested (**Supplemental material appendix C**).

The questionnaires were completed after a regular patient (and parent) outpatient visit for CD follow up. The questionnaires were filled out by the parents if the child was younger than 12 years or by the parents and child together if the child was older than 12. Since the visit was a regular medical check for CD, coeliac-specific antibodies in the serum were tested according to (inter-)national CD guidelines[22,23]. The Coelkids study protocol was approved by the Medical Ethical Committee of LUMC and by the respective boards of each participating center and complied with Good Clinical Practice guidelines (registration in the Dutch Trial Register, NTR3688, www.trialregister.nl).

Data management and statistics

The responses to the questionnaires were entered by the participants themselves into a secure web-based data management application (NEN7510 certified). Patient characteristics such as age, age at CD diagnosis and sex were recorded, as was their serum TG2A at the time of questionnaire completion. Pearson's Chi-square test for trend and unpaired t-test and one-way ANOVA were used where appropriate. Cohen's kappa was used to measure inter-rater agreement for the two questionnaires. A two-tailed probability of $p < 0.05$ was considered significant. Analyses were performed with SPSS software (version 20.0, IBM Corp. Armonk, New York).

Results

In total, 151 of 165 children and young adults completed both questionnaires on dietary compliance. Patient characteristics are shown on **Table 1**.

Table 1 *Characteristics of the 151 participating children and young adults with coeliac disease (CD)*

Age (years), median (IQR)	10.2 (7-14)
Age groups, no. (%) 2-6 years 7-10 years 11-15 years >16 years	 27 (18%) 53 (35%) 39 (26%) 32 (21%)
Female, no. (%)	100 (66%)
Age at diagnosis of CD (years), median (IQR)	3.8 (2-7)
Duration of CD (years), median (IQR)	5.3 (3-8)
Anti-tissue transglutaminase type 2 antibodies (TG2A) measured, no. (%)	145 (96%)
TG2A positive*, no. (%), Mean TG2A level if positive, in U/mL (range)	14 (10%) 20.6 (7-56)
Being on another diet in addition to gluten-free diet, no. (%)	9 (6%)
Other family members present with CD, no. (%)	45 (30%)
Expected complaints after eating gluten containing food, no. (%)	39 (26%)

* Cut-off of normality >7 U/ml

The results of the short questionnaire and the dietary interview questionnaire do not correlate with each other since dietary adherence problems (scores 1 and 2) were reported by 52% (n=78) and 14% (n=21) of the patients when using the dietary interview questionnaire and the short questionnaire, respectively (Cohen's kappa 0.034) (**Table 2**).

Table 2 *Inter-rater agreement between the short gluten-free dietary adherence questionnaire and dietary interview, tested in children, adolescents and young adults with coeliac disease, measured by Cohen's kappa.*

Dietary compliance		**Short questionnaire**			
		Score 1 (non- adherent)	**Score 2 (adherent but with errors)**	**Score 3 (strictly adherent)**	**Total**
Standardized interview	**Score 1 (non- adherent)**	11	0	6	17
	Score 2 (adherent but with errors)	3	0	58	61
	Score 3 (strictly adherent)	6	1	66	73
	Total	20	1	130	151

Cohen's kappa 0.034

TG2A were measured in 145 patients wherein 10% of them turned out to be positive. Sex and age at the time of questionnaire completion were similarly distributed among the patients with positive and negative TG2A levels (64% and 66% female, p = 0.87; and 10.6 and 11.0 years, p = 0.79, respectively). However, patients with positive TG2A were significantly older at the time of diagnosis and had been treated with a GFD for a shorter period of time when the questionnaires were filled out (6.5 and 4.3 years, p = 0.017, and 4.1 and 6.8 years, p = 0.042, respectively). Positive TG2A results were mostly seen in children 2-6 years of age as well as among adolescents and young adults >16 years (both groups 15%). As shown in **Table 3**, only the results of the short GFD questionnaire were significantly associated with the presence of a positive TG2A, which was found in 35% (n=6) of the patients with self-reported noncompliance (n=17) versus 6% of the patients who reported good adherence (n=8 out of 127 patients, p=0.003).

Table 3 *Distribution of anti-tissue transglutaminase type 2 serum antibodies (TG2A) levels in 145 coeliac patients according to the results obtained from the gluten-free diet (GFD) adherence questionnaires.*

Short dietary questionnaire†	**Number of patients**	**TG2A positive* patients Number**	**%**	**p-value (Fisher's exact test)**
1 Non adherence	17	6	35	
2 Errors	1	0	0	p = 0.003
3 Good adherence	127	8	6	
Standardized dietary interview†	**Number of patients**	**TG2A positive* patients Number**	**%**	**p-value (Fisher's exact test)**
1 Non adherence	14	3	21	
2 Errors	59	6	10	p = 0.191
3 Good adherence	72	5	7	

* Cut-off normality >7 U/ml

† Score of 1 reflecting non-adherence to a GFD, 2 reflecting adherence to a GFD but with errors that require correction, and 3 reflecting strict adherence to a GFD.

Older age was the only factor significantly associated with noncompliance, with mean ages of 15.5 , 11.5 and 10.1 years for patients who were completely non-adherent to the diet, those who adhered but committed errors, or those who strictly adhered, respectively (p<0.001). Compliance to the GFD diet was best in children younger than 6 years of age, with strict dietary compliance (score 3) in 74% of them, and without any child

in the totally non-adherent group (score 1). Sex, age at CD diagnosis and the presence of other family members with CD did not influence GFD compliance (data not shown), nor did being on another diet in addition to the GFD (data not shown) or the presence of complaints after gluten ingestion.

Since the results of the short questionnaire did not correlate with that of the dietary interview questionnaire, we modified the latter to become more comprehensive and user-friendly. To achieve this, the items of the dietary interview questionnaire were separately weighed using item-total correlation, with regard to their contribution to the score. This resulted in reduction of the total number of items from 26 to 11. For reproducibility and verification, this modified questionnaire was tested in the 158 coeliac children and young adults of the eHealth intervention group of Coelkids, who also completed the short diet questionnaire and the dietary interview questionnaire. Sex, age and disease duration at time of questionnaire completion were similar in the Coelkids intervention and control group: 69 and 66% female, 11.0 and 11.4 years and 6.9 and 6.7 years, respectively. As shown in **Table 4**, there was a moderate correlation between the results of self-reported dietary adherence as assessed by the dietary interview questionnaire and the adapted questionnaire with 11 items (Cohen's kappa 0.56). All patients who reported to be totally non-adherent the GFD by using the dietary interview questionnaire (n=14) also reported as non-compliant upon completing the adapted 11-item questionnaire. However, discrepancies in self-assessed dietary adherence were observed in 24% of the patients (n= 38).

Tabel 4 *Inter-rater agreement between the long gluten-free diet adherence questionnaire, tested in children, adolescents and young adults with coeliac disease, and its modified gluten-free diet score, measured by Cohen's kappa.*

Dietary compliance		**Modified score**			
		Score 1 (non-adherent)	**Score 2 (adherent but with errors)**	**Score 3 (strictly adherent)**	**Total**
Standardized interview	**Score 1 (non- adherent)**	14	0	0	14
	Score 2 (adherent but with errors)	0	35	22	57
	Score 3 (strictly adherent)	0	16	71	87
	Total	14	51	93	158

Cohen's kappa 0.56

Discussion

Although GFD is the only effective therapy for CD, guidelines that assess dietary adherence do not exist for either adults or children. By using a standardized dietary interview questionnaire in this study, we found a high percentage (52%) of children, adolescents and young adults who followed the GFD, but they did so with errors or did not follow the diet at all. In 40% of these coeliac patients, dietary transgressions would have been unnoticed if dietary adherence were only assessed using CD-specific serology and the short dietary adherence questionnaire validated in adults. This short questionnaire has proven to have a good correlation with serum levels of coeliac-specific antibodies in adults[13] and in children during their first year of starting a GFD[24]. Our results however show that patients may have negative TG2A serology, yet do not strictly adhere to the diet when using the long questionnaire derived from the dietary interview. This discrepancy between GFD adherence and results of CD serology has been previously demonstrated, both for EMA and TG2A[9,25,26] and indicates that measurement of CD-specific antibodies is not a sensitive tool to detect problems in dietary adherence[24]. One of the main differences between the short GFD adherence questionnaire and the dietary interview questionnaire is that the latter addresses practical issues possibly leading to errors related to the GFD such as storing gluten-free products at home separate from gluten-containing food, and preparing gluten-free food with separate kitchen utensils. These issues address the actual daily risk of gluten consumption. Despite our efforts, we were unable to reduce the length of the dietary interview questionnaire. However, with the increasing use of electronic patient records and eHealth tools, completing questionnaires before or during a medical consultation should be easily implemented in the health care for children and young adults with CD. In addition, we expect that this will contribute to improvement of care for CD patients by, on the short term empowering them, leading to a better diet adherence, and possibly, on the long-term, by avoiding complications of their disease. If a better dietary assessment will also result in improvement of symptoms can only be investigated in (future) prospective studies.

One of the strengths of this project is the prospective design and the relatively large group of patients included. All participants completed the questionnaires and TG2-serology was available in 96% of them. Our cohort seems representative for CD children and young adults, including the decreased dietary compliance in adolescents; an established fact in CD populations[8,27].

One may argue that since the dietary interview questionnaire was designed with the purpose of this project in mind, without previous validation, it might not accurately demonstrate problems with GFD compliance. The questionnaire, however, was developed by an experienced dietitian who has worked extensively with coeliac patients. It

therefore reflects the patient encounter and contains the same questions asked during this dietetic consultation. Furthermore, completing the standardized dietary questionnaire by patients avoids omissions that may occur during face-to-face consultations, for example, due to insufficient time. A possible limitation could be the fact that we also included CD patients who had been on a GFD for only 1 or 2 years. In our opinion, the first year on a GFD is intense and essential to learn how to adhere to the diet (with regard to shopping, reading etiquettes, contamination etc). Therefore, face-to-face contact seems to be more adequate than questionnaires. One could argue that CD specific serology might not have normalised in the first 1-2 years on a GFD, thus impeding the correlation between serology and the questionnaires. However, it has been shown that TG2A normalisation occurs after 12 months on a GFD in the majority of children[28], therefore limiting this effect.

Recent developments in diet monitoring include a new method of measuring gluten immunogenic peptides in the stool and urine[29,30], which seem to enable direct and quantitative assessment of gluten ingestion. However, its future role needs to be more extensively evaluated because result manipulation by gluten avoidance prior to testing is possible since gluten immunogenic peptide analysis only detects gluten if ingested a few days before testing.

In conclusion, our results show that dietary adherence should be assessed by a dietary interview in combination with specific CD antibodies determination. Available short dietary questionnaires and TG2A serology alone do not detect all errors in GFD adherence in children and young adults. A standardized dietary questionnaire reflecting the regular dietary interview as performed by an experienced dietitian is a good alternative to face-to-face contact.

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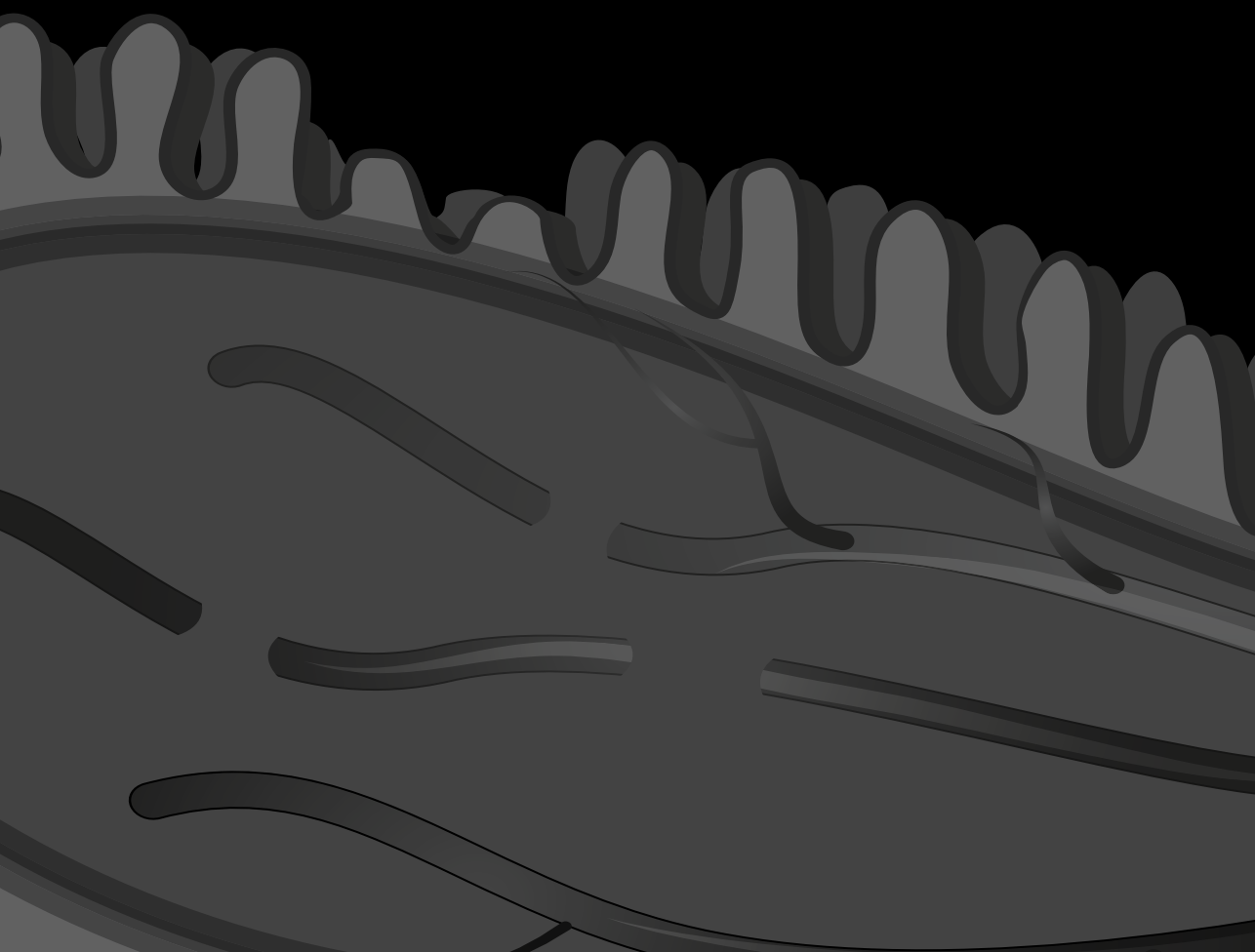
PART II

RISK GROUPS



CHAPTER 4

Impact on parents of HLA-DQ2/DQ8 genotyping in healthy children from coeliac families

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Wessels MMS

Vriezinga SL

Koletzko S

Werkstetter K

Castillejo-De Villasante G

Shamir R

Hartman C

Putter H

Pal van der SM

Wijmenga C

Bravi E

Mearin ML

Abstract

Due to the association of coeliac disease and HLA-specificities DQ2 and DQ8, HLA-typing can be used for risk determination of the disease. This study was designed to evaluate the knowledge of parents from coeliac families regarding HLA-typing and the impact of HLA-typing on the perception of the health of their children. A structured questionnaire was sent to the Dutch, Spanish and German parents participating with their child in the European PreventCD study on disease prevention in high-risk families, addressing parents' understanding of, and attitude towards HLA-typing, distress related to HLA-typing and perceived health and health related quality of life of their children. 68% Of parents of 515 children returned the questionnaires with 85% of children being DQ2/DQ8 positive. The majority of all parents answered the questions on knowledge correctly. 48% Of parents of DQ2/DQ8 negative children thought their child could develop coeliac disease. More distress was reported by parents of DQ2/DQ8 positive children ($p<0.001$). All parents showed few regrets and would repeat HLA-typing in future children. Perceived health and health related quality of life were similar.

CONCLUSION We can say that misinterpretation of DQ2/DQ8 negative results by parents is frequent. DQ2/DQ8 positive results do not affect perceived health and health related quality of life of children, but may cause temporary negative feelings among parents. Parents of coeliac families seem to support HLA-typing.

Introduction

Coeliac disease (CD) is the most common intolerance to a dietary component in children and adults[1]. In genetically predisposed individuals, CD is precipitated by the ingestion of gluten, which are storage proteins in wheat (gliadin), rye (secalin) and barley (hordein)[1,2]. T-cells in the lamina propria of the small intestines recognize the gluten peptides when they are bound to the Human Leukocyte Antigen (HLA) class II specificities -DQ2 and/or -DQ8 on antigen presenting cells[2]. Screening for CD can be done by measuring CD specific antibodies against the enzyme tissue transglutaminase type 2 (anti-TG2A), endomysium (EMA) and deamidated gliadin peptides[3]. CD is treated with a gluten-free diet. Long term complications of untreated CD are among others diarrhea, abdominal pain, perinatal problems, osteoporosis, and cancer[1,4].

CD has a strong genetic component, since 90% of CD patients carry the class II HLA-DQ2 haplotype, about 5% the HLA-DQ8 molecule[5-8] and the rest usually the half of the HLA-DQ2 heterodimer.

The HLA-DQ2 and DQ8 haplotypes are present in over 25% of the general population[6], but only 1% actually develops CD1. This indicates that HLA-DQ2 and -DQ8 haplotypes are necessary, but not sufficient for disease development. Around 40% of the heritability of CD is explained by HLA-DQ2 and/or -DQ8. First degree family members of CD patients who are HLA-DQ2 and/or DQ8 positive (DQ+) have an increased risk of approximately 10% to develop CD[9]. Because of the high negative predictive value of HLA-typing for development of CD, unnecessary invasive investigations (i.e. blood punctures, duodenal biopsies) in HLA-DQ2 and DQ8 negative (DQ-) individuals can be avoided. Consequently, pediatricians can offer HLA-typing to first-degree family members of a child diagnosed with CD[10].

Being diagnosed with CD may have impact on patients' Health Related Quality of Life (HRQoL)[11,12]. Currently, concerns are raised about the impact on parents of genetic testing for chronic diseases which only provide a crude estimate of disease risk. It is known that the attitude of parents towards genetic testing for common preventable health conditions in their children is moderately positive[13]. Unfavorable results may cause parental concerns about the lifelong increased risk for development of CD and the bond between child and parents might be affected with the development of a sense of vulnerability and protectiveness of the child, despite the child's good health[14]. To our knowledge, the parental impact of HLA-typing for CD risk determination has not been studied. Our aim was to investigate the effect on parents from families with high risk for CD of HLA-typing in their healthy children. We expected parents whose children are DQ+ to take on a more adversary position towards HLA-typing. Subsequently, we hypothesized that parents of

DQ+ children will interpret more of their children's symptoms as a sign of CD, and will have a higher rate of health care utilization.

Patients and Methods

For this descriptive study we took advance of the Dutch, Spanish (Reus) and German cohorts of the ongoing European family study investigating the prevention of CD, PreventCD (www.preventcd.com). The PreventCD study investigates the possibility of reducing the frequency of CD by introducing small quantities of gluten to infants, between 4-6 months of age, preferably while they are being breast-fed. In families with a diagnosed CD member, newborns were HLA-typed shortly after birth and if they were positive for HLA-DQ2, DQ8 or half of the DQ2 allele, they were considered HLA-DQ+ and included in the study (hereafter referred to as PreventCD children)[15]. After informing the parents, the result of the HLA-typing was sent as a letter with an individual risk score: no risk if DQ-, 10% risk if DQ+. In addition, HLA-typing was offered to parents and siblings of the PreventCD children.

For this study, the parents were asked to fill in a questionnaire in their native language after receiving the HLA-typing results. Parents of children who had developed CD prior to sending the questionnaire were not invited to participate. Parents attending the hospital in Israel with their in PreventCD participating children were also asked to complete the questionnaire. Since no information could be obtained about the Israeli participation rate, the results on these children and parents were not used in the main analysis.

Questionnaires

Most questions were weighed on a Likert scale.
Parents' knowledge on HLA-typing for CD was assessed by the following true-false questions: 1. All children that have HLA-DQ2 and/or DQ8 will develop CD (incorrect) and 2. A healthy person can be carrier of HLA-DQ2 and/or DQ8 (correct). Good knowledge was defined as answering both correctly. To assess parents' understanding of the HLA-typing results of their child, they were asked to evaluate its risk to develop CD.

Since there was no HLA-Impact questionnaire available, we constructed one consisting of a form based on validated questionnaires concerning genetic testing for hereditary blood diseases[16] and cystic fibrosis[17]. The attitude of the parents towards HLA-typing was evaluated by asking them about the reliability, possible regrets and whether they would consider HLA-typing in case they would have another child . Furthermore, parents rated the information they received on HLA-typing on a scale from 1 ("very bad")

until 10 ("excellent"). Parents' behaviour after receiving the HLA-typing results of their children was assessed by the frequency of discussing the results with friends or family and by the methods used to get additional information on HLA-typing, e.g. internet, patient association et cetera. Parental feelings after receiving the HLA-results were assessed by 5 questions concerning the degree of worries, anxiety, unhappiness, reassurance and relieve. A total score reflecting these feelings (min 0-max 5, high score indicating positive feelings) was calculated. To assess the parental mood in the week prior to the questionnaire we used a 4-item Hospital Anxiety and Depression Scale (HADS)[18] subscale, a high score corresponding with a positive mood. Internal consistency for all scales was evaluated with Cronbach's alpha.

Perceived health of the children was assessed by questions about the frequency of diarrhoea, vomiting, abdominal distension, constipation and fatigue in their child in the last 3 months. Parents were asked about the concerns about their children's health, and how often their children had been ill in comparison with other children. The health care utilization was assessed by the frequency of medical visits of the child during the last month. Frequent consultation was defined as 2 or more visits monthly.

Health related quality of life (HRQoL) of the children was evaluated with the validated TNO-AZL Preschool Children Quality of Life Questionnaire (TAPQOL) 43-item version[19].

Data management and analysis

Parents in the Netherlands, Spain and Germany received an e-mail with an invitation to participate accompanied by a personal digital code, which gave access to the online questionnaire. A paper version was also available if requested. They were also asked to fill in the questionnaire about their HLA-typed children not participating in PreventCD, but only with regard to the child specific part. The online questionnaire was designed as a web form in a secure data management application ProMISe, (www.msbi.nl/promise), with automatic data export to the Statistical Package for Social Sciences (SPSS) version 20.0.

Scale reliability of the HADS items and the negative feelings after receiving the HLA-typing result were calculated with SPSS. For statistical analysis of the HLA-Impact questionnaire, the Pearson's Chi-square test for trend, the unpaired t-test, Kruskall-Wallis and Mann-Whitney U test were used where appropriate. For the TAPQOL, a score ranging from 1 to 100 was calculated for every domain, a higher score reflecting a better HRQoL in the relevant scale[19, 20]. Due to abnormal distribution of the TAPQOL results, we used the Mann-Whitney U test for statistical analysis of the results. For each questionnaire item, a difference was found significant if $p < 0.05$.

This study was approved by the Medical Ethics Committees of all the participating centers.

Results

Table 1 *Distribution of characteristics of children and parents and of parental attitude and knowledge of HLA typing and CD.*

	Netherlands	Germany	Spain	Total
Children (nr)	214	181	91	486
Participation (%)	85	62	59	72
Age children (mean, years)	3.2	4.1	5.0**	3.8
Sex children (% male)	49	53	52	51
Questionnaire filled in by mother (%)	88	89	74	86
Educational level parents high* (%)	82	81	65	79
Children HLA DQ2 and/or DQ8 positive (%)	86	80	93	85
Time between HLA-result and questionnaire (mean, years)**	2.4	2.8	4.4**	2.8
Knowledge of HLA-typing: Both questions answered correctly (%)	97**	87	92	92
No regrets about HLA-typing (%)	98	100	97	99
Parents would repeat HLA-typing in future children (%)	98	97	94	97
Perception of given information about HLA-typing as good (%)	89	86	67	85
Searched for extra information about HLA-typing (%)	31	38	39	34

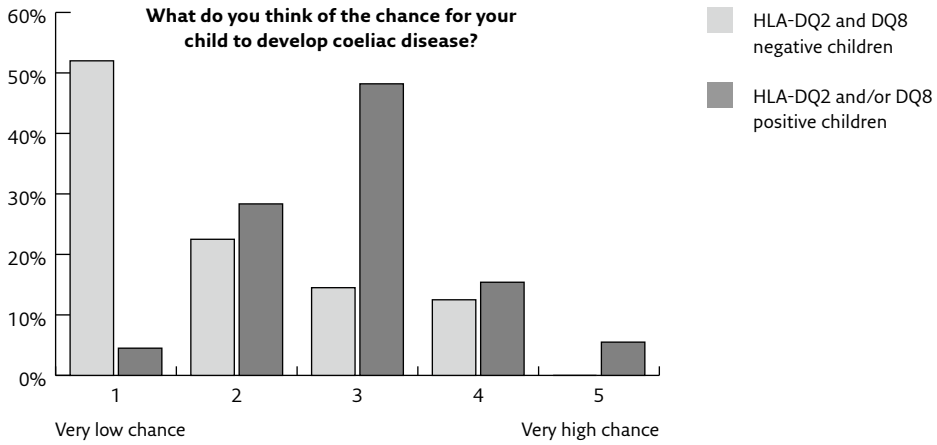
* Defined as: low — vocational education; intermediate — General Certificate of Secondary Education (GCSE)/A-level; high — (under)graduate/post-graduate

** $p<0.05$

The characteristics of the participating children and parents are presented in **Table 1**. The age, sex, parent completing the questionnaire, parental educational level and time between receiving the HLA-typing result and completing the questionnaire were similar among the HLA negative and positive children (data not shown).

Both parents of DQ+ and DQ-children had a good knowledge on HLA-typing in CD (88% and 93% respectively) and they found HLA-typing a "reliable" test (88% and 93% respectively). However, when asked about the chance of their own child to develop CD according to his/her HLA-typing, 48% of the parents of the DQ- children thought their child still had a chance to develop CD (**Figure 1**). There was no correlation between this perception by the parents and their opinion about the reliability of the HLA-test (data not shown).

Figure 1 *Parental assessment of risk of developing coeliac disease in their child according to HLA-typing results.*



No parents of DQ- children had regrets about HLA-typing and all of them would perform HLA-typing in their child if they would have one later on. Likewise, almost all parents of DQ+ children (99%) did not regret HLA-typing in their children and 97% of them wanted HLA-typing in their child if they would have one in the future. The information about the HLA-typing was similarly appreciated by the parents of DQ- and DQ+ children with scores of 8.1 and 8.0 respectively on a 1-10 scale. The majority of parents found the information on HLA-typing to be "sufficient, clear and complete" (76% and 87% of parents of DQ- and DQ+ children respectively). Parents who received unfavourable results looked for more information on HLA-typing (38% vs. 17%, $p = 0.01$), with the Internet mentioned most frequently. HLA-typing results were discussed equally with family and friends in both groups (57 and 67% DQ- and DQ+ respectively). The knowledge of HLA typing and CD, the attitude towards HLA-typing and the information about it among the different countries is shown in **Table 1**.

Significantly more negative feelings were reported by parents who received unfavourable HLA results (mean 3.4, SD 0.9) compared with those receiving favourable results (mean 4.6, SD 0.6) ($p<0.001$). The German parents experienced more negative feelings than the Dutch and Spanish parents in the same situation (mean score 3.0 (SD 0.8) compared to 3.5 (SD 0.7) and 3.7 (SD 1.0) respectively, $p<0.001$). Stressful feelings of the parents at the time of completing the questionnaire were similar, irrespective of the HLA-typing result, (both groups mean score 3.2; SD 0.7 favourable and 0.5 unfavourable result), but in Spain, parents receiving unfavourable results reported more stress than parents in the Netherlands and Germany (mean 2.8 (SD 0.6) vs. 3.4 (SD 0.5) and 3.1 (SD 0.5) respectively, $p<0.001$).

Consultation of a doctor in the month prior to filling in the questionnaire was reported equally among the DQ- (37%) and DQ+ (38%) children, with 8% reporting a frequent consultation in both groups. The majority of the parents of DQ- (63%) and DQ+ (65%) children found the frequency of illness in their child similar to the one in the general population. However, significantly more parents of DQ+ children (21%) had concerns about their children's health compared to parents of DQ- children (10%) (p=0.022). When asked about specific health complaints of their children during the 3 months prior to filling in the questionnaire, these were similarly distributed among the DQ- and DQ+ children, with diarrhoea in 42% and 48% respectively, vomiting in 25% in both groups, abdominal distension in 19% and 26%, constipation in 23% and 30% and fatigue in 25% and 32% respectively. There were no significant differences between the countries regarding the perceived health of the children by their parents (data not shown).

Health Related Quality of Life (HRQoL)

This was analysed in 299 children aged 9 months to 6 years using TAPQOL. HRQoL was similar for all domains in the DQ- and DQ+ children with the exception of a lower score in DQ+ children with regard to the domain concerning problem behaviour.

Discussion

The results of this study, the first to assess the impact of HLA typing on families at risk for the development of CD, show a positive parental perception of HLA-typing in their children and that unfavourable results indicating risk for CD do not lead to a lower HRQoL in their offspring. It also demonstrates that despite good knowledge about HLA-typing for CD, almost 50% of parents of a negative tested child think that this child has a chance to develop CD. These results are relevant, since over the years, HLA-typing has become more frequently used in the diagnostic process of CD and it is part of the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) diagnostic guidelines on CD[10].

The fact that half of the parents of children who tested negative misinterpreted the results of their child is remarkable, since the majority of these parents knew that their child could not participate in PreventCD due to no risk of CD. This pessimistic interpretation by the parents of a favourable result of genetic testing has been reported before after testing for cancer genes in children[21]. One explanation could be the failure of adjustment to the sudden removal of the disease scenario, as it has been found in genetic testing for Huntington disease[22]. The above mentioned misinterpretation of negative HLA findings opens the question whether parents correctly understand the results of HLA-typing for CD and of susceptibility genetic testing in general[23]. Paradoxical risk interpretation of genetic testing has also been reported in newborn screening for type 1 diabetes[14], with 10% of mothers

over- or underestimating the risk. It is also known that processing statistical information is often difficult for people and that interpretation of results may be dependent on several factors, such as personal and cultural opinions[24]. This outcome should prompt physicians to make sure that parents understand the results and to improve the way of giving information to the parents, for example with a short information brochure about genetic testing. This could be helpful, especially since we found that parents who received favourable results were less inclined to look for additional information on HLA-genotyping and CD.

As expected, parents who received unfavourable HLA results experienced more negative feelings. On the other hand, regrets about HLA-typing were scarce and did not influence the parents' willingness to repeat HLA-typing in future children. Our results show that HLA-typing results do not influence the HRQoL of children as perceived by their parents. However, although the overall concern about the health of their children was low, HLA positive results lead to more parental concerns in general, not being supported by more health care utilization or specific health complaints in the DQ+ group. One possible explanation is that parents in our study are participating in a research project where they are frequently interviewed about the health of their children. Cultural differences with regard to genetic testing did not seem to be taking place, since similar results were seen in the different European countries. Data on parents of 46 children from Israel, who filled in the questionnaires on paper during visits to the hospital, are consistent with this finding. Our study has the strength of novelty and of having been done in a unique, international, prospective cohort of families with high risk of CD. Possible limitations of the study include that the validity of the tools used to assess the parental knowledge of and attitude towards HLA-typing has been not tested in other populations, since they were created for this study. Scale reliability, however, was tested and showed good scores. The question raises whether our group of parents, with a high educational level characteristic for people participating in research projects, is representative for CD families in general. On the other hand, if high educated parents misinterpreted HLA-typing results, it is likely that CD parents in general will do so even more. The vast majority of the participating parents were mothers, reflecting their role as being the primary caregivers in most families. Given this fact, we cannot completely exclude that fathers may be less positive than mothers about HLA-typing in CD. Since the participating parents in this study were participants in the PreventCD project, one could argue that these parents are mostly people with a positive attitude towards screening for CD and HLA-typing, and that our conclusions may not be supported by the parents of CD in general. However, the large size of the PreventCD cohort may be considered as representative for young children from families with high risk for CD[15]. Our study group also has a relatively small number of DQ- children, but this is characteristic for families with high risk for CD, as shown before[9].

Conclusion

Parents of CD families support HLA-typing for genetic risk determination for CD. Unfavourable HLA results cause temporary negative feelings in the parents, but no increased health concerns about their children. The interpretation of HLA-DQ2 and/or HLA-DQ8 negative results of their own children is difficult for the parents despite good knowledge of CD and HLA-typing in general. This should urge us to provide parents with good and easy to understand information on this topic.

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CHAPTER 5

Towards an individual screening strategy for first degree relatives of coeliac patients

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Wessels MMS

Rooij de N

Roovers L

Verhage J

Vries de W

Mearin ML

Abstract

Coeliac disease (CD) is known to be more prevalent in first-degree relatives of patients. In this retrospective cohort study of 609 relatives between 1994 and 2016, we investigated the effect of sex, HLA-type and age at time of index coeliac diagnosis. Pearson's Chi-square test and Kaplan-Meier survival analysis were used as statistical analyses. CD screening was carried out for 427 relatives (70%), resulting in a prevalence of 15%. HLA-typing in 335 relatives showed HLA-DQ2/DQ8 positivity in 87.5%. In 63% of children and all parents, coeliac disease was diagnosed at first screening. It was diagnosed significantly more often in females, HLA-DQ2 homozygosity, and children (all $p<0.05$). In children aged 0-1 year at time of index diagnosis, coeliac disease was diagnosed after consecutive screening in 58%, after 3.9 + 2.5 (max 10) years ($p<0.001$).

CONCLUSION Future screening policies for relatives of coeliac patients should include retesting, especially in HLA-positive relatives younger than 10 years of age. In addition, one-time coeliac specific antibody testing alone could be sufficient to rule out the disease in adolescent siblings and parents of newly diagnosed coeliac patients.

Introduction

Coeliac disease (CD), which can develop at any age, is a chronic, immune-mediated disease in which alterations occur in the mucosa of the small intestine induced by ingestion of gluten in genetically predisposed individuals[1]. Gluten are storage proteins in wheat (gliadin), rye (secalin) and barley (hordein)[2]. The diagnosis of CD is made through detection of the presence of a variable combination of gluten-dependent clinical manifestations, CD specific antibodies, HLA-DQ2 and/or HLA-DQ8 haplotypes and enteropathy[1]. Serological testing for CD is possible through detection of IgA class transglutaminase type 2 antibodies (TG2A), endomysium antibodies (EMA) or antibodies against deamidated gliadin peptides[1,3]. CD can be successfully treated with a life-long gluten-free diet, which restores small bowel histology and clinical complaints in most cases[4]. CD is found to occur in 1% of the general population[5]. It is often unrecognized, which can be partially explained by the variable clinical presentation, from diarrhea, weight loss and abdominal pain, to nonspecific signs and symptoms such as fatigue, osteoporosis, iron deficiency anaemia and no symptoms at all, referred to as silent CD[1]. Later in life, untreated CD can lead to an increased risk of osteoporosis and even cancer[2,6].The disease is multifactorial, and one in which genetics plays an important role. In 90-95% of coeliac patients the HLA-DQ2 haplotype is identified, with HLA-DQ8 being present in most of the remaining patients. Both haplotypes occur in 30-40% of the general population, which indicates that these haplotypes are necessary, but not sufficient, for developing CD[1]. As already demonstrated in many studies, first-degree relatives (FDRs) of coeliac patients are at a higher risk of developing CD than the general population, with a prevalence of CD in FDRs varying from 2.6-11.9%[7-16]. Therefore, the Dutch and European CD guidelines recommend CD screening in individuals at risk of developing CD, such as FDRs[1,3]. In FDRs without the HLA-DQ2 and/or DQ8 haplotypes, the chance of developing CD is nil, so follow-up through further CD investigations can be omitted[3]. On the other hand, FDRs who carry the HLA-DQ2 and/or DQ8 haplotypes, have an increased risk of approximately 10% of developing CD[16-18]. Thus, since CD is a condition that can evolve at different stages in life, repeated serologic tests for CD can be necessary in HLA-DQ2 and/or DQ8 positive FDR's[15,19]. Several studies have shown that the risk of developing CD among FDRs is influenced by multiple factors, such as age, sex, relationship with the index patient and HLA-genotype[11,16,20,21]. However, CD guidelines do not give guidance about the frequency of CD screening and duration of follow-up needed in HLA-DQ2 and/or DQ8 positive FDRs.

The aim of this study in FDRs is to investigate the effect of sex, HLA-type and age at time of CD diagnosis in the index coeliac patient, in order to establish a better screening protocol for these high risk individuals.

Methods

Study design and participants

A historic cohort in the Rijnstate Hospital in Arnhem, the Netherlands, included mothers, fathers and siblings of all 174 consecutive pediatric CD patients (up to 18-years) from 1994 until January 2016. After 2012, two CD-specialised gastroenterologists in our hospital started to refer offspring to the pediatric gastroenterologist, therefor 24 children (10 female) of 16 adult biopsy proven CD patients were also included between 2012 and January 2016. All pediatric coeliac diagnoses were based on ESPGHAN diagnostic criteria and all patients were seen at least once by succeeding pediatric gastroenterologists with a special interest in CD[1,22]. In parents, CD diagnosis was based on a combination of positive CD specific serology and Marsh > 2 duodenal lesions. FDRs were identified using the electronic patient record system, where detailed descriptions of the family setting are registered. Cross-check was done by identifying individuals living at the same address as the coeliac patient in order not to overlook FDRs. The FDRs were categorized in groups according to their age at time of coeliac diagnosis in the index patient: group 1: 0-1 year, group 2: 2-5 years, group 3: 6-10 years, group 4: 11-24 years, group 5: >25 years. Groups 1-4 represent the siblings and children of coeliac patients and group 5 represents the parents of the index coeliac children.

According to (inter)national guidelines, screening was offered to all FDRs. Follow-up of FDRs was also discussed. If parents wanted follow-up screening in their (other) HLA-DQ2 and/or DQ8 positive children annual or biannual visits were planned with screening of at least EMA combined with TG2A in most cases. Standard follow-up was not advised to parents themselves.

Since we focussed on FDRs and their specific risk of developing CD, relationship to the CD index patient was recorded. Also dated CD-specific serology, HLA-typing results (when performed) and diagnostic duodenal biopsies were recorded. In FDRs the follow-up duration until eventual CD diagnosis was defined as the time between diagnosis of the index patient and CD diagnosis in the FDR. Total follow-up duration was defined as the time between diagnosis of the index relative of a FDR and the time of analysis in February 2016. HLA-typing results were considered as unknown if no HLA results were found in the electronic patient record, as negative if negative for DQ2 and DQ8, and as positive if positive for DQ2 and/or DQ8. HLA-DQ2 and/or DQ8 positive results were categorized according to the risk of development of the disease[11,16,18] into a high, intermediate and low risk group as defined in **Table 1**.

Table 1 *HLA risk group classification*

HLA risk group	Haplotypes
High-risk	DQ2DR3/DQ2DR3, DQ2DR3/DQ2DR7
Intermediate-risk	DQ2DR3/DQ7DR5, DQ2DR7/DQ7DR5, DQ2DR3/DQ8DR4, DQ2DR3/other*, DQ2DR7/DQ2DR7, DQ2DR7/other
Low-risk	DQ2DR7/DQ8DR4, DQ8DR4/DQ8DR4, DQ8DR4/DQ7DR5, DQ8DR4/other, DQ7DR5/DQ7DR5, DQ7DR5/other

* Other: refers to any HLA-DQ haplotype except DQ2DR3, DQ2DR7, DQ8DR4 or DQ7DR5.

Statistical analysis

Pearson's Chi-square test, unpaired t-test, Mann-Whitney U test and Kaplan-Meier survival analysis were used where appropriate. For comparison, a log-rank test was used stratified according to sex, HLA risk group or age group. For each item, a difference was found significant if $p < 0.05$. The data was analyzed in version 21.0 of the IBM Statistical Package for the Social Sciences (SPSS).

Medical ethical consideration

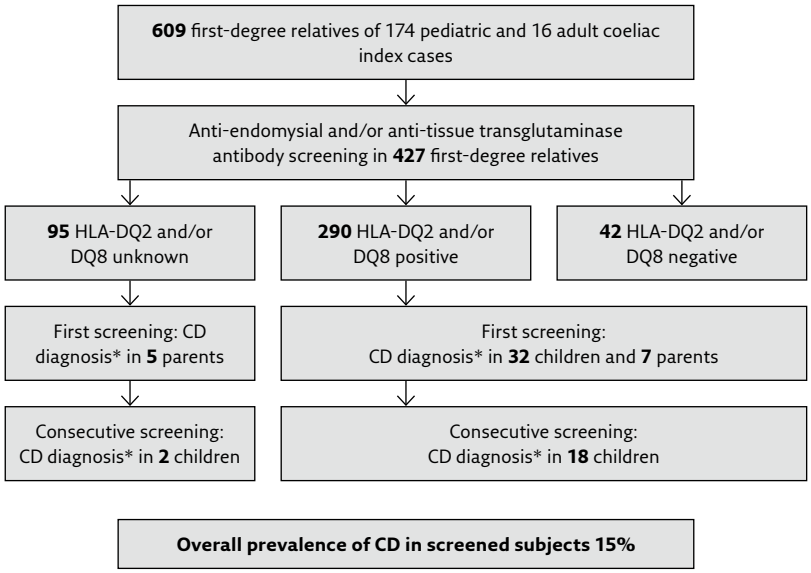
The procedures followed were in accordance with the ethical standards of the Medical Research Involving Human Subjects Act and the principles of the declaration of Helsinki (59th General assembly, Seoul, October 2008) of the World Medical Association. Formal approval from the local feasibility committee of Rijnstate Hospital Arnhem was obtained.

Results

A total of 609 FDRs were identified, for which it was found 70% (n=427) had been screened for CD (205 parents, 181 siblings and 41 offspring). The reasons for not performing screening in the other 182 FDRs were not known. The overall prevalence of CD in the screened subjects was 15% (64/427). In 30% of all cases, CD was diagnosed after the initial screening. The participant flow is shown in **Figure 1**.

Table 2 shows the characteristics of the 427 screened FDRs with regard to sex, relationship to the index patient, age at time of diagnosis of CD in the index patient, HLA risk group and follow-up duration. Significantly more females were diagnosed with CD (61%,

Figure 1 *Flow chart of participants (first-degree relatives of coeliac patients)*



* Coeliac disease (CD) diagnosis in children based on ESPGHAN diagnostic criteria, CD diagnosis in parents based on combination of positive CD specific serology and Marsh 2-3 duodenal lesions.

p=0.031), however this gender effect was observed only in sisters and not in mothers and daughters of CD index patients (**Table 2**). HLA-typing was performed in 332 FDRs and 12.7% of them were found to be HLA-Q2 and/or DQ8 negative and therefore not at risk for CD.

Among the 290 FDRs who were HLA-DQ2 and/or DQ8 positive, CD was diagnosed in 29% of the children (34 siblings and 16 offspring) and 6% of parents (3 mothers and 4 fathers), with a mean follow-up duration after CD diagnosis in the index patient of 2.7 years (SD ± 3 years). In 18% of the siblings and offspring, diagnosis was established without duodenal biopsies according to the latest ESPGHAN criteria because there were symptoms suggestive of CD[1]. In all parents, CD was diagnosed based on duodenal biopsies except in one mother, who was both TG2A and HLA-DQ2 positive and had resolution of symptoms after starting a gluten-free diet.

Table 2 *Characteristics of the 427 first-degree relatives of celiac patients with screening of coeliac disease (CD).*

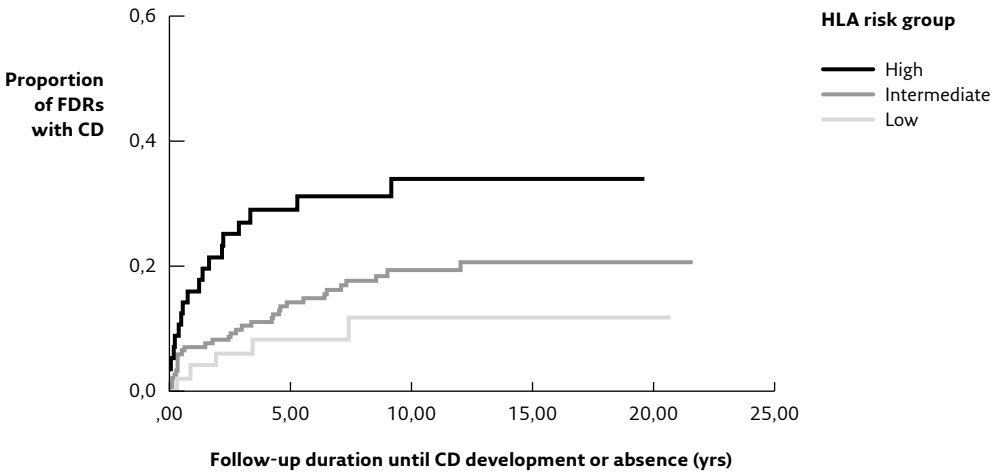
Characteristic	CD +	CD -	HLA-DQ2 and/or DQ8 -
Number of subjects	n = 64	n = 321	n = 42
Age at diagnosis of CD in index patient – years			
Children, p=0.67	4.0 ±4.2	4.3 ±4.7	3.4 ±4.1
Parents, p=0.30	34.8 ±4.2	36.3 ±5.1	34.5 ±4.4
Sex – Female, %, p=0.031			
Relation of FDR to index patient – %, p<0.001	61	47	62
Mother	11	26	38
Father	9	27	14
Sister	39	18	22
Brother	16	22	22
Daughter	11	3	2
Son	14	4	2
HLA risk group – %, p<0.001			
High	28	12	NA
Intermediate	52	47	NA
Low	8	14	NA
Unknown	12	27	NA
Mean follow-up duration in years until CD development or end of follow-up†			
Children, p<0.001	3.1 ±3.4	8.8 ±5.1	NA
Parents, p<0.001	1.5 ±1.7	9.9 ±5.3	NA

† Mean follow-up duration until CD development or not = time between CD diagnosis in the index patient and CD diagnosis in the FDR and/or the time of the study (January 2016).
NA: Not applicable

As shown in **Table 2**, the intermediate-risk HLA genes (DQ2DR3 heterozygosity and DQ2DR7 homozygosity) were the most prevalent, both in FDRs who were diagnosed with CD and those who were not (52% and 47% respectively). In contrast, high-risk HLA genes (DQ2 homozygosity) were significantly more common in FDRs who were diagnosed with CD (28% versus 12% in the FDRs without CD, p=0.001). The Kaplan-Meier survival analysis according to HLA risk group in **Figure 2** shows that FDRs with high risk HLA genes were diagnosed significantly earlier than those in the intermediate or low risk groups (p=0.011). In 90% of the high risk FDRs, CD was diagnosed within 4 years of the diagnosis of the coeliac index case (13 at first screening and 3 during follow-up) compared to 80% and 75% in the intermediate and low risk group respectively.

In the 95 FDRs in whom HLA-typing had not been performed (noticeably more parents than children: 59% vs 33% respectively), CD was diagnosed in 3 mothers and 2 fathers

Figure 2 *Coeliac disease (CD) diagnosis according to HLA risk group by means of Kaplan-Meier survival analysis*



and in 2 siblings, all based on positive CD specific serology and Marsh 3 duodenal lesions. There were no differences with regard to sex and mean follow-up time to CD diagnosis between FDRs with and without performed HLA-typing (data not shown).

Table 3 *Correlation between the age of the first-degree relative (FDR) at time of index coeliac diagnosis and the age of the FDR at own coeliac diagnosis.*

	Children				**Parents**
Age groups	**0-1 yr n = 90**	**2-5 yrs n = 52**	**6-10 yrs n = 45**	**11-24 yrs n = 17**	**25-48 yrs n = 181**
CD diagnosis (n)	24 (27%)	12 (23%)	11 (24%)	4 (24%)	13 (7%)
CD diagnosis at first screening (represented as % of CD diagnoses)	42	90	72	100	100
Mean follow-up duration until CD diagnosis (Q1-Q3 Tukey Hinges)	3.9 (1.9-5.4)	2.8 (0.2-5.2)	2.6 (0.2-6.4)	0.6 (0-1.1)	1.5 (0.5-1.9)
Follow-up duration without CD diagnosis (Q1-Q3 Tukey Hinges)	9.4 (6.1-12.2)	10.1 (5.2-15.1)	6.5 (2.4-10.8)	7.4 (0.5-10.7)	9.9 (5.8-13.9)
Mann-Whitney U test with regard to follow-up duration, p-value	<0.001	<0.001	0.003	<0.001	<0.001

* SD= standard deviation

** Mean follow-up duration in years without CD diagnosis until analysis in February 2016

Table 3 shows the significant association between the age of the FDR at time of coeliac diagnosis in the index patient and the identification of CD after the first screening ($p<0.001$), with young children being diagnosed after a longer follow-up period than older children and adults. Siblings and offspring were significantly more often diagnosed with CD when compared to parents of coeliac patients (25% and 7% respectively, $p<0.001$).

In total, CD was diagnosed at first screening in 63% of the children and in all the parents (**Table 3**). The youngest group (0-1 years) had the lowest CD identification rate at first screening (42%), while all CD cases were identified within 10 years of follow-up. All children aged 2-5 years were diagnosed at first screening, except for one sister, aged 2.2 years at the time of CD index diagnosis, who was diagnosed at the age of 7.1 years (**Table 3**). In children aged 6-10 years, only 2 siblings were not diagnosed during the first screening (sister of 6.7 years and brother of 6.8 years of age at the time of index diagnosis, diagnosed during follow-up at 12 and 14.8 years respectively). Both siblings had complaints suggestive of CD, being the reason for the renewed follow-up screening. All other coeliac children in this age group were identified during first screening. In the adolescent group (11-24 years) all coeliac cases were identified during first screening. In the majority of parents (61%), first CD screening was done within 1 year after diagnosis in the index patient, in 3 parents (23%) after 1-2 years and in 2 parents (15%) after 4.0-5.6 years.

Discussion

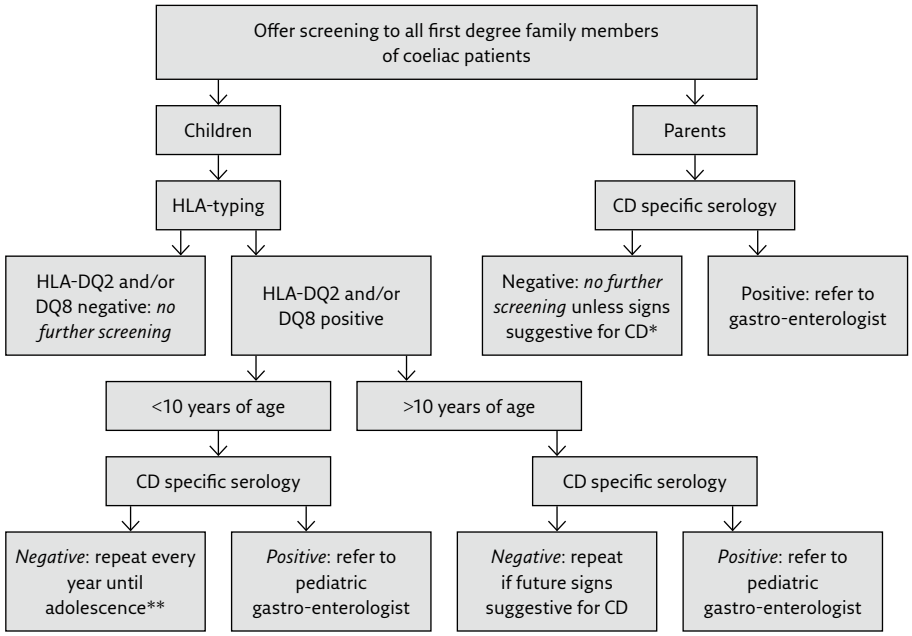
This retrospective cohort study in families of coeliac patients substantiates the higher prevalence of CD found in FDRs, which in our study was 15% after screening. Our data show a higher rate of CD in siblings and offspring when compared to parents of CD patients, as demonstrated by previous studies and a recent meta-analysis[8,21,23]. Again, as previously demonstrated, we found a higher prevalence of CD in sisters of CD patients[21]. In agreement with the results of prospective studies in birth cohorts of FDRs, we have found a significantly higher prevalence, at a younger age, in children who are HLA-DQ2 homozygous[11,24].

Our results suggest that the timing of CD specific antibody testing could be individualized depending on the relationship of the FDR with the index patient and her/his age at time of the index diagnosis. Since all CD cases in adolescents and parents were detected during first screening, further follow-up screening might not be necessary in this age category. Although there were 2 parents with a longer interval between the coeliac diagnosis in the index patient and their own diagnosis, diagnoses in these cases were still the result of the first screening. The reason for this delay could not be retrieved from the patient records, but might be due to the fact that screening was left to the discretion of the parents.

Our findings with regard to CD diagnosis in parents of coeliac patients are in accordance with a Swedish cohort of FDRs who were retested 20-25 years after first CD screening because of newly diagnosed CD in the family[25]. Only 2 new cases of CD were found, with one of these FRDs already having mild enteropathy 20-25 years earlier. On the other hand, our findings support the fact that repeated screening is necessary in offspring of CD patients and siblings younger than 10 years of age in order to be able to diagnose CD. Due to the retrospective nature of our study we can only indicate that repeated screening for CD beyond the age of 10-12 may not be necessary. All children in our cohort who were diagnosed during adolescence, were either adolescents at the time of first screening or had a long period between the first and follow-up screening.

The strength of our study lies in the fact that we have studied a large group of FDRs. The percentage of CD found in our cohort (15%) was similar to the percentages found in other studies[11,21,26] so the results appear representative of coeliac FDRs in general. This

Figure 3 *Screening algorithm for family members of (newly) diagnosed coeliac patients*



* Consider HLA-typing and referral to gastroenterologist in case of negative CD specific antibodies but signs suggestive for coeliac disease: consider gluten challenge and reinvestigation.

** During adolescence: repeat CD specific serology in case of future signs suggestive for coeliac disease

is supported by the distribution of HLA-types found in our cohort which is similar to other cohorts described in the literature[11,16,24], even though the percentage of HLA-DQ2/DQ8 negative FDRs in our study (12.5%) was somewhat lower than described before (14-21%)[11,27,28].

One possible limitation is the retrospective cohort study design. After initial screening of CD, which was done in 70% of FDRs, CD specific antibodies were not tested on a regular basis, since it was left to the FDRs/parents whether follow-up took place[23]. A stringent repetitive screening policy in FDRs might have led to an even higher prevalence of CD than found in our study, therefore stressing further the importance of follow-up. Prospective studies with regular screening of FDRs are needed to be able to develop a tailored and effective screening strategy for CD in FDRs. In the meantime, we propose an algorithm that can be used, preferably within the first months after coeliac diagnosis (**Figure 3**).

Since family members tend to have a lower gluten containing diet when compared to the general population, one has to bear in mind that negative serology in HLA-DQ2 and/or DQ8 positive FDRs can lead to unjust reassurance. In those cases, gluten challenge with repeated serology and duodenal biopsies are justified.

Conclusion

Screening of FDRs of coeliac patients in a clinical setting revealed a prevalence of CD of 15%. Repeated testing of CD specific serology in HLA-DQ2 and/or DQ8 positive siblings and offspring, younger than 10 years of age at the moment of CD diagnosis in the index patient, is necessary to diagnose CD as early as possible. This should be continued until at least early adolescence (10-12 years of age) and is especially true in HLA-DQ2 homozygous siblings of coeliac patients. In addition, one-time CD specific antibody testing could be sufficient to diagnose CD in siblings who are adolescents at the time of diagnosis in the index patient, and parents of newly diagnosed coeliac children. Our results may contribute to developing future recommendations for CD screening frequency and follow-up duration of relatives of coeliac patients.

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CHAPTER 6

Raising the cut-off level of anti-tissue transglutaminase antibodies to detect coeliac disease reduces the number of small bowel biopsies in children with type 1 diabetes — a retrospective study

SUBMITTED

Wessels MMS*

Velthuis A*

Lochem van E

Duijndam E

Hoorweg-Nijman E

Kruijff de I

Wolters VM

Berghout E

Meijer J

Boksma JA

Mul D

van Alfen-van der Velden JA

Mearin ML

Setten van P

* Contributed equally

Abstract

OBJECTIVE Our aim was to study the optimal cut-off value for the anti-tissue transglutaminase type 2 IgA antibody in serum (TG2A) to select for diagnostic small bowel biopsies for coeliac disease (CD) in children with type 1 diabetes mellitus (T1DM). In particular, we endeavour an increase in specificity and positive predictive value and more importantly a decrease in normal histology, without losing too much sensitivity.

PATIENTS AND METHODS Children with T1DM who had both elevated TG2A titers during regular screening and duodenal biopsies during the course of their diabetes were included. Anti-endomysial antibodies (EMA) if present were recorded. The optimal TG2A cut-off value was determined using receiver operating characteristics (ROC) curve analysis; and compared with the cut-off value used in the ESPGHAN guidelines in terms of sensitivity, specificity, positive and negative predictive value. TG2A titers were expressed as the ratio between the value obtained and the upper limit of normal (ULN).

RESULTS A total of 63 children were included. The optimal cut-off value for performing a biopsy proved 11xULN. Raising the cut-off value from 3xULN to 11xULN changed the sensitivity from 96% to 87%, increased the specificity from 36% to 73%, the positive predictive value from 88% to 94% and the negative predictive value from 67% to 53%. The percentage of normal histology was reduced from 12% to 6%.

CONCLUSION Our data indicate that increasing the TG2A cut-off value for performing duodenal biopsies in children with T1DM and suspected CD leads to a substantial reduction of unnecessary biopsies. We advocate to adapt the ESPGHAN 2012 guidelines for this group of children, including monitoring patients with TG2A levels below 11xULN over time.

Introduction

Children with type 1 diabetes mellitus (T1DM) are at risk of developing coeliac disease (CD). Both conditions are autoimmune diseases showing strong linkage to the human leukocyte antigen (HLA) system[1]. The prevalence of CD among patients with T1DM is estimated between 3 to 10%[2]. Most children with both T1DM and CD are asymptomatic or present with non-specific symptoms[3]. Duodenal biopsies is the gold standard for diagnosis of CD in children with T1DM[3]. CD is not only believed to cause diminished diabetes control in children with T1DM, but may also result in complications, including decreased bone density and gastrointestinal malignancies[1]. CD is treated with a gluten-free diet (GFD)[4]. Therefore, children with diabetes are regularly screened for CD; at diagnosis of T1DM and subsequently every 1-2 years thereafter[5]. Anti-tissue transglutaminase type 2 IgA antibodies (TG2A) are commonly used for screening and have a sensitivity and specificity above 90%[6]. Despite this high accuracy, the interpretation of the TG2A titers in children with T1DM has proven difficult. Significant quantitative differences exist among different TG2A assays[7], elevated TG2A titers often show spontaneous normalization in children with T1DM[8] and people at genetic risk for CD (like children with T1DM) have more often false-positive TG2A results[9]. In 2012, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) introduced new guidelines for diagnosing CD[10], including an algorithm for asymptomatic children belonging to a high-risk group, like children with T1DM. In this algorithm, the cut-off value of serum TG2A titers for performing biopsies in these children is 3x upper limit of normal (ULN). This study was prompted by our observation that biopsies performed in children with T1DM and TG2A titers >3xULN are often not consistent with CD. We hypothesize that the cut-off value of 3xULN was chosen too low. The aim of our study was to investigate the optimal cut-off value for the TG2A titers in order to overcome negative biopsies in children with T1DM without losing too much sensitivity.

Patients and methods

Study design and settings

This is a retrospective observational study covering the time period 2002-2015. Data were collected both at University Hospitals and middle to large secondary care clinics: Leiden University Medical Center (LUMC) Leiden, University Medical Center Groningen (UMCG), Rijnstate Hospital Arnhem, Haga Hospital The Hague, Children's Diabetes Centre Nijmegen (CDCN), Maas Hospital Pantein Beugen, Spaarne Hospital Hoofddorp, St. Antonius Hospital Nieuwegein, Zuwe Hofpoort Woerden, MC Zuiderzee Lelystad, Isala Hospital Zwolle, Deventer Hospital and University Medical Center Utrecht (UMCU). Due to the retrospective nature of this study, informed consent was not required. The pro-

cedures followed were in accordance with the ethical standards of the Medical Research Involving Human Subjects Act and the principles of the declaration of Helsinki (59th General assembly, Seoul, October 2008) of the World Medical Association. Formal approval from the local feasibility committee of Rijnstate Hospital Arnhem was obtained.

Study group

The study population consisted of all consecutive children and adolescents (<19 years of age) with T1DM, who underwent esophagogastroduodenoscopy with duodenal biopsies because of elevated TG2A titers>3xULN[10]. Screening with CD specific serology in these children was done every 1-2 years after diagnosis of T1DM according to the ISPAD international guideline for the management of pediatric T1DM, regardless of symptoms[5]. Exclusion criteria were: IgA deficiency, a GFD at the time of duodenal biopsies, CD diagnosed before T1DM and an interval >180 days between measurement of the TG2A titer and duodenal biopsies.

Data collection

Data was retrieved from either patient charts or electronic data systems and entered into standard forms using Research Manager version 5.2.0.5 (Cloud9 Health Solutions,the Netherlands). Clinical, anthropometric and laboratory data were collected. This included several baseline characteristics such as age at first duodenal biopsies, gender, other autoimmune disease(s), family history of CD and other autoimmune diseases and GFD adherence. Presence of symptoms suggestive for CD[10] was also registered: chronic or intermittent diarrhea[11], failure to thrive, weight loss, delayed puberty, amenorrhoea, iron-deficiency anaemia, chronic abdominal pain, chronic constipation, chronic fatigue, dermatitis herpetiformis-like rash and spontaneous fracture/osteopenia.

Serology

Since 13 different hospitals participated in this study, TG2A titers were assessed while using different types of assays, different arbitrary units and different cut-off values. In order to compare the TG2A titers, results were expressed as the ratio between the value obtained and the upper limit of normal. This ratio was rounded to whole numbers. When a TG2A level was written in the file as >50, it was regarded as 50 and >128 was regarded as 128, etcetera. Since some patients were analyzed twice (accompanied by a second biopsy), without starting a GFD in between, the total number of measurements exceeds the total number of patients included in our study. TG2A IgA ELISA's used were obtained from 6 different manufacturers: Aesku (Wendelsheim, Germany), n=4; Euroimmun (Lübeck, Germany), n=2; Inova (San Diego, California), n=4; Orgentec (Mainz, Germany), n=3; Phadia (Freiburg, Germany) n=50; Sanquin (Amsterdam, The Netherlands), n=2. Anti-endomysial antibodies (EMA) as determined by indirect immunofluorescence were recorded in all patients.

Duodenal biopsy

All children included in this study underwent esophagogastroduodenoscopy in order to obtain duodenal biopsies, 4 from duodenum and 1-2 from bulb. Histological findings were revised and classified according to the Marsh criteria[12] by a single pathologist, specialized in Marsh typing. This pathologist was blinded for previous interpretations and clinical and laboratory findings. Definite CD was confirmed by Marsh 2 or 3 histology in combination with the already known positive coeliac serology. CD autoimmunity without histology alterations in the small bowel biopsies was considered potential CD[10].

Statistical analysis

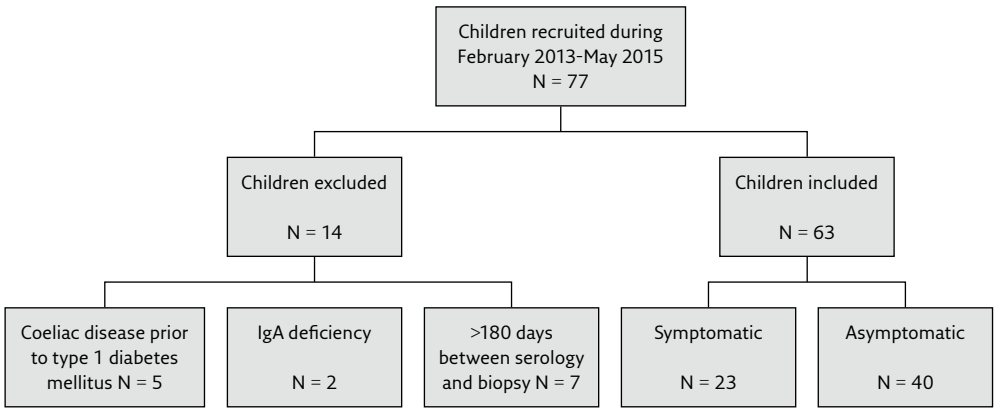
Continuous data are presented as mean ± standard deviation (SD). Differences in means between groups were tested using the Students' T-test. Categorical data are presented as frequencies and percentages. Differences in percentages between groups were tested using the Pearson chi-square test or Fisher's Exact test. Receiver Operating Characteristic (ROC) analyses were performed to determine the optimal TG2A cut-off value. Sensitivity, specificity, positive and negative predictive values were calculated. Sensitivity and specificity were tested by the McNemar test. Statistical analysis was performed using IBM SPSS Statistics (version 18.0). A P-value less than 0.05 was considered to indicate statistical significance.

Results

Study design

In our study, 77 children were eligible. A total of 63 children fulfilled our inclusion criteria. 14 children were excluded (**Figure 1**). The median interval between TG2A measure-

Figure 1 *Flowchart of participants (children with type 1 diabetes mellitus).*



ment and duodenal biopsies was 72 days (range 0-178). The total number of analysed TG2A titers was 65, since 2 patients underwent a second paired antibody determination as well as second biopsies.

Baseline characteristics

The baseline characteristics of all patients are presented in **Table 1**. In our study population, a preference for females was noticed (62%). Symptoms suggestive for CD were present in 37% of the patients. When comparing asymptomatic with symptomatic children, no statistically significant differences were observed in baseline characteristics, neither in associated autoimmune disease, family history of associated autoimmune disease, family history of CD nor in Marsh histology. Three children suffered from autoimmune hypothyroidism. The prevalence of CD in the total group is 83%, with a higher prevalence in the asymptomatic patients when compared to the symptomatic children, 88% and 74% respectively (p>0.05).

Table 1 *The baseline characteristics and differences between the asymptomatic and symptomatic children with type 1 diabetes mellitus and elevated tissue transglutaminase antibodies.*

	Study population n=63	**Asymptomatic children n=40**	**Symptomatic children n=23**	**P-value**
Mean age in years (at time of 1st biopsy)	9.7 (SD 4.7)	10.5 (SD 4.2)	8.2 (SD 5.1)	0.06
Female n(%)	39 (62)	27 (68)	12 (52)	0.23
Other autoimmune disease n(%)	3 (5)	3 (8)	0 (0)	0.31
Family history of other autoimmune disease n(%)	21 (33)	13 (33)	8 (35)	0.85
Family history of coeliac disease n(%)	4 (6)	2 (5)	2 (9)	0.62
CD (Marsh 2-3) n(%)	52 (83)	35 (88)	17 (74)	0.31

Analysis of cut-off value

ROC-curve analysis showed that the optimal TG2A cut-off value for performing a biopsy in children with T1DM, as calculated by ROC-curve analysis, is 11xULN. This cut-off value provides a sensitivity of 87% and a specificity of 73%. The cut-off value of 3xULN, described and advised in the latest ESPGHAN guideline, results in our study in a sensitivity of 96% and a specificity of 36%. **Figure 2** shows the ROC-curve, **Table 2** shows the coordinates of the curve, with sensitivity and 1-specificity.

Figure 2 *Receiver operating characteristics curve to determine the optimal tissue transglutaminase antibodies cut-off value for performing diagnostic biopsies for coeliac disease in type 1 diabetes mellitus.*

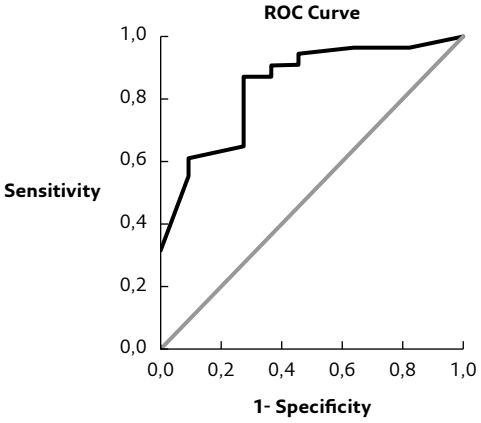


Table 2 *Coordinates of the receiver operating characteristics curve to determine the optimal tissue transglutaminase antibodies cut-off value for performing duodenal biopsies.*

Cut-off value (xULN)	Sensitivity	1-Specificity
1,0	1,000	1,000
2,0	0,963	0,818
3,0	0,963	0,636
5,0	0,926	0,455
7,0	0,907	0,455
9,0	0,907	0,364
10,0	0,870	0,364
11,0	0,870	0,273
13,0	0,852	0,273
15,0	0,648	0,273

Changing the cut-off value of TG2A: consequences for clinical practice

Table 3 illustrates the different positive predictive values (PPV) for small bowel mucosal atrophy, depending on different cut-off values for normality (CoN), from 3xULN to 11xULN.

PPV for CoN increases from a minimal of 89% at 3xULN to a maximum of 94% at 11xULN with NPV of 67% and 53% respectively. Raising the CoN from 3xULN to 11xULN results in a decrease in sensitivity from 96 to 87% (p=0.06) and an substantial increase in specificity from 36 to 73% (p=0.13). Overall, a CoN of respectively 3 and 11xULN resulted in 12% (7/59) and 6% (3/50) normal (false positive) duodenal biopsies.

EMA as additional test

EMA was negative in only 3 cases, who all had Marsh 0-1 histology. Adding EMA positive results did not improve the PPV of increased TG2A levels for villous atrophy, since 50% of EMA positive patients had normal duodenal histology.

Table 3 *Diagnostic performance of different cut-off values of normality of tissue transglutaminase antibodies*

TG2AxULN	Number of children biopsied	Diagnosed with CD	Sensitivity (%)	Specificity (%)	Positive predictive value for CD (95% CI interval)	Negative predictive value for CD (95% CI interval)
>1.0	65	54	100	18	85.7 (82-89)	100
>3.0	59	52	96	36	88.7 (83-92)	66.7 (29-91)
>5.0	55	50	93	55	90.9 (84-95)	60 (34-82)
>10.0	51	47	87	64	92.2 (84-96)	50 (31-70)
>11.0	50	47	87	73	94.0 (86-98)	53.3 (34-71)
>13.0	49	46	85	73	93.9 (85-98)	50 (32-68)
>15.0	38	35	65	73	92.1 (81-97)	29.6 (20-41)

Discussion

With this study in T1DM children, we have shown that it is justified to increase the specificity and PPV by increasing the current TG2A cut-off value for performing diagnostic biopsies for CD. In order to have the highest NPV, we believe it is best raised to 11xULN and not to 10xULN even though the latter is commonplace for pediatricians vice versa not to perform duodenal biopsies in symptomatic children. Our data underline the fact that the choice of the cut-off of >3xULN for performing diagnostic biopsies in children with T1DM detected by screening, as part of the diagnostic algorithm for asymptomatic risk groups in the 2012 ESPGHAN guidelines for CD[10], was not based on evidence, but on

consensus aiming to avoid unnecessary biopsies. We decided not to exclude the symptomatic children, who were also found in our study population, since the TG2A screening was done during regular, planned visits rather than due to symptoms. In our cohort, 55% of the children with Marsh 0-1 histology were also "symptomatic", which may reflect the fact that the symptoms are non-specific for CD. Our results are in line with those of several recent studies in children with T1DM showing that TG2A levels varying from 5-8 to 10xULN were stronger predictive of villous atrophy than >3xULN[7,8,13,14]. While screening tests usually optimize sensitivity to find new patients[15], specificity is the parameter that needs optimization to avoid false-positive results. The latter is in our opinion crucial in T1DM children suspected of CD. First because endoscopy is an important burden for children who already have a chronic disease and omitting biopsies means avoiding the risks of anaesthesia in these children. Second, unnecessary biopsies should be prevented from a cost-effective point of view[15].

In our opinion, the decrease in sensitivity by increasing the CoN of TTGA to >11xULN is acceptable, since normalization of elevated TG2A can occur in up to a third of the asymptomatic T1DM patients on a gluten containing diet[16], allowing clinicians to wait and see first in these patients. In this recent retrospective study in Israel of newly diagnosed children with T1DM, high TG2A levels (>10x ULN) at diagnosis and three months thereafter, were predictive of CD[16]. Furthermore, if normalization does not occur and CD diagnosis is established with duodenal biopsies after all, this does not seem to have short term adverse effect either on diabetes regulation or on bone mineral density[17,18]. In our cohort, EMA did not contribute to the question whether or not to perform diagnostic biopsies. EMA negativity seemed to point to normal histology, but we were not able to draw a definite conclusion due to the small number of EMA negative patients, so future (prospective) studies are needed to sort this out.

Since children with T1DM with positive CD serology but normal duodenal histology can be considered potential CD patients, regular monitoring of CD serology is warranted. Recommendations on treatment and frequency of follow-up in potential CD are lacking, but since development of active CD is described in a third of the patients on a gluten containing diet[19], follow-up is important. In patients with T1DM and potential CD, young age and persistent positive TG2A over time seem to increase the risk of transfer into active CD[16]. In our cohort, we also found a tendency towards younger age in the group that was diagnosed with CD (p=0.06). Our result indicate that withholding biopsies is acceptable in children with T1DM and low TG2A titers if serology is being followed over time. Other studies, that show spontaneous normalisation[8,20] or fluctuation[13,17] of CD specific antibodies support our finding that low TG2A titers should be followed over time without performing immediate duodenal biopsies. Since children with T1DM need medical checks on a regular basis, assessment of TG2A every 6-12 months is in our opinion feasible. Performing CD

specific serology only in symptomatic children is not advised, since we have found an even higher prevalence of CD in asymptomatic patients when compared to symptomatic children, 88% and 74% respectively. Other studies report varying results. In one study, 71% of children with both T1DM and CD reported no gastrointestinal symptoms[21], while in another study, 76% of children with both conditions had at least 1 gastrointestinal symptom[22].

One of the strengths of our study is that this is the first to perform ROC-curve analysis to determine the TG2A cut-off value for performing biopsies in children with T1DM. Furthermore, biopsies were revised by a single pathologist specialized in Marsh typing. For this reason, histological examination was not affected by interobserver variability[23]. In addition, this is as far as we know, the first and largest study in type 1 diabetic children revising the performance of the revised ESPGHAN diagnostic criteria for CD[10].

The limitations of our study relate to its retrospective character and the sample size. Furthermore, we have compared TG2A measured with different types of assays. In the absence of an international standard, expressing the outcome in multiples of the ULN is currently the best option which has been used in several studies. Hopefully, in the near future an international standard will be introduced. It has been stated that comparison based on multiples of the ULN are valid[24], because studies have shown acceptable agreement between most second-generation kits[25,26]. Several other studies[26,27] have used multiples of ULN to compare data from different manufacturers. ROC-curve analysis in which only data from Phadia, Aesku and Euroimmun (chosen because they showed good agreement in xULN in Table A from the ESPGHAN guidelines[10]) were included, resulted in the same ROC-curve (data not shown).

In conclusion, we have shown that raising the current cut-off level of TG2A to 11xULN in children with T1DM to perform duodenal biopsies results in a 50% decrease of false positive and thus unnecessary biopsies. In diabetic children with TG2A levels lower than 11xULN, no diagnostic biopsies should be performed, but serological follow-up on a gluten containing diet should be done.

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PART III

DISCUSSION

CHAPTER 7

General discussion and conclusion

Although coeliac disease (CD) is a frequent, but still underdiagnosed disease, focus in research should not only be on diagnostics and novel therapies, but also on best ways to take care of and monitor patients once they are on a gluten-free diet (GFD). In addition, evidence based screening policies in populations at risk in order to diagnose CD as early as possible should be developed as secondary prevention of the disease. The questions formulated at the start of this thesis with regard to these 2 domains are presented in **Table 1**, together with the assembled recommendations.

Table 1 *Main conclusions of this thesis*

Questions	Findings	Recommendations
Do nutritional deficiencies persist or develop in coeliac children after start of a GFD?	Nutritional deficiencies recover within 1 year of GFD.	Standard blood-investigations besides CD specific serology are not necessary after one year of follow-up.
Do short GFD questionnaires detect infrequent dietary transgressions in coeliac children?	The short dietary questionnaire developed by Biagi does not provide more information than CD-specific serology.	The standardized dietary interview, especially if completed before a face-to-face consultation, provides detailed information on dietary (non-) compliance.
What is the impact of HLA-screening in children at risk for coeliac development?	Parents of young children from coeliac families support HLA-typing and would repeat it in future children.	HLA-typing should be offered to children from risk families with the associated information provided.
What is the best screening method in FDRs of newly diagnosed coeliac patients?	One time screening could be enough in adolescent siblings and parents of newly diagnosed coeliac patients.	Regular screening by means of CD-specific serology should be offered to all HLA-DQ2 and/or DQ8 positive pediatric FDRs <10 years of age.
When should duodenal biopsies be performed in children with T1DM and elevated TG2A serology, since serology is often found to be false positive and/or declining spontaneously in these children?	In asymptomatic children with T1DM, 12% of the children have normal duodenal mucosa when biopsied in case of a TG2A titer of >3xULN.	Follow-up of serology instead of performing endoscopy to retrieve biopsies in these patients seems safe and appropriate.

In-depth discussion of the findings and recommendations

Until present, the GFD is the only treatment of CD. Although it has a proven positive effect on the health of the coeliac patient, effective long-term management programs are lacking for children as well as for adults. The need for effective long-term follow-up to improve compliance with the diet and outcome of coeliac patients has been recognized by many expert groups[1–5], since delay of the GFD appears to lead to an increased risk of co-morbidity, mortality and tendency to a lower quality of life[6,7]. Therefore, in

2016, evidence-informed expert recommendations were published for the management of CD in children by pediatric gastroenterologists from the United States of America[8], in which the shortage of good quality data regarding this matter was emphasized. At present, standard medical care for CD children consists of regular visits to the pediatrician or pediatric gastroenterologist to evaluate overall health, anthropometrics, GFD adherence and laboratory investigations including CD specific antibodies and additional tests to rule out deficiencies and co-morbidity. With this in mind, it is important to acknowledge earlier reports that have indicated that follow-up care is not being provided to all patients, both in the pediatric as adult population[9-12]. In a pediatric cohort in Israel, it was shown that patients lost to follow-up have a poorly controlled disease with more non-adherence to the diet and positive CD-specific serology[13]. One can only speculate whether this non-compliance leads to more long-term complications, since follow-up data are lacking, both in adults as well as in children, and in untreated as well as treated patients. It is therefore indeed relevant for the establishment of evidence based follow-up care of CD patients treated with a GFD.

This was the reason to study eventual nutritional deficiencies that may occur in CD patients and that are usually checked during follow-up. These alterations, although often present at diagnosis, disappear within one year of GFD, as we have shown in **Chapter 2**. This means that standard blood-investigations besides CD specific serology are not necessary after one year of follow-up. This outcome is important, due to its consequences for the organization of the health care for children with CD, because blood tests are time-consuming and expensive, and in a few children also painful and frightening. The percentages of nutritional deficiencies found in our study were comparable with previous studies, with the exception of vitamin B12, which was much lower in our cohort (2%) in comparison to earlier studies in adolescents and adults (12-41%)[14-17]. Our findings on the frequency of thyroid dysfunction (nearly 4%) are similar to the ones from previous studies, with the prevalence of thyroid autoimmunity (elevated thyroid stimulating hormone (TSH) or presence of thyroperoxidase (TPO) antibodies), hypothyroidism and hyperthyroidism varying from 10-26%, 2-6% and 1%, respectively[18,19]. The rationale behind thyroid function testing as part of a CD patient's follow up is based on the high frequency of thyroid autoimmunity in CD[20], but there is conflicting evidence about the GFD's protective effect in the development of auto-immune thyroid disease[21-23]. Based on our results, routine testing of TSH, commonly used to screen for thyroid disease, should be discouraged, since (temporarily) abnormal results occur often without abnormal FT4 levels and thyroid disease. This can lead to overdiagnosis and anxiety in patients and parents. Thyroid testing should therefore be reserved for symptomatic children, presenting with abnormal growth or pubertal development, fatigue, altered defecation and appetite, muscle aches or tremor, ophthalmopathy, thermodysregulation and altered school performance. If tested, FT4 levels should be determined. Since

mucosal healing after start of a GFD tends to behave similarly in adults and children, we hypothesize that the same advice could be given to coeliac adults, but there is no current evidence to support this.

The next step enabling us to evaluate the management and follow-up of children with CD is defining the best way to assess dietary adherence, which is the only available treatment. Since the diet is not always easy to follow, identifying the patients who do and do not comply to the diet is vital. While an extensive dietary evaluation by a trained dietitian is considered the best method to evaluate GDF adherence, this method is time-consuming, taking 20-30 minutes per patient, and requires expert personnel. In **Chapter 3**, we have shown that a standardized dietary questionnaire is a good alternative to the face-to-face contact with a dietitian. A short questionnaire developed and tested in adults[24], did not provide more information on diet adherence than anti-tissue transglutaminase type 2 antibodies (TG2A). Both do not detect all errors in children and adolescents with CD. We have pointed out a decreased dietary compliance in adolescents, an established fact in CD populations[25-27]. Sex, age at CD diagnosis and the presence of other family members with CD did not influence compliance, nor did being on another diet besides the GFD or the presence of complaints after gluten ingestion. Despite our efforts, we were unable to reduce the length of the dietary interview. However, with the increasing use of electronic patient records and eHealth tools, completing questionnaires before or during a medical consultation should be easily implemented in the health care for children and young adults with CD. The routine use of this dietary questionnaire especially when completed before the face-to-face contact, in combination with TG2A determination, will facilitate the communication between patients/parents and doctors, with a better focus on pitfalls and problems with the GFD. It will help the doctor to have an insight into possible dietary transgressions and reasons why and when they occur. This will provide the opportunity to anticipate on possible educational counselling and support. We expect that this will contribute to improvement of care for CD patients by, on the short term, empowering them leading to a better diet adherence, and possibly on the long-term, by avoiding complications of their disease. Not only will it be a useful tool in daily practice, but the dietary interview can also be used in prospective studies looking at long-term outcome of CD patients on a GFD. Novel methods of measuring gluten immunogenic peptides (GIP) in urine and in faeces can add value to diet monitoring[28,29]. GIP enables direct and quantitative assessment of gluten intake. It can help to detect incidental dietary transgressions that are not detected by CD specific serology and to identify patients non-compliant with the diet. However, because GIP analysis only detects gluten ingested a few days prior to testing, gluten ingestion before this time may remain undetected. GIP determinations might also be helpful in patients who adhere to the diet, but who have persistent elevated TG2A. If TG2A is still declining and GIP is negative on repetitive basis, reassurance of patients and their parents is probably justified.

Another step forward to improve health and quality of life in coeliac patients is to diminish the level of under diagnosis. To be able to do so, awareness of the disease and an increased level of suspicion, both among doctors and the general public, is important. In addition, secondary prevention by early diagnosis and treatment should be further improved by developing screening programs for risk groups. When addressing screening for CD, it is important to look at the benefits of the outcome first of all. Looking at the literature, there is some evidence for screening strategies as a method of preventing complications and reducing medical costs[30-32]. However, benefits and cost-effectiveness of screening remain controversial[33,34]. Active case finding can be considered, albeit well known that the use of symptoms to identify CD patients has its limits. As it happens symptoms associated with CD are as prevalent in individuals with and without the disease[35]. However, case finding programs in children based on symptoms are an alternative for general screening programs, which is opposed to by the Medical Ethical Committees in the Netherlands. Since health benefits after diagnosis and treatment are expected in symptomatic children, permission to perform the GLUTENSCREEN study in the youth health care in the province North-Holland in the Netherlands was granted. Screening for CD in certain high risk groups is recommended both by the Dutch and European CD guidelines[2,36], as individuals with other autoimmune diseases such as type 1 diabetes mellitus (T1DM), autoimmune thyroid and liver disease, individuals with syndromes like Down, Turner and Williams syndrome and with selective IgA deficiency and also first degree relatives (FDRs) of coeliac patients have a higher risk of getting the disease. In order to achieve better care for high risk groups, involvement of general practitioners in the Netherlands is imperative when updating coeliac guidelines, since their own current guideline on CD does not advice to screen FDRs[37], who mostly are under medical attention of the general practitioner.

Because of the high negative predictive value of HLA-typing for CD, unnecessary investigations in HLA-DQ2 and DQ8 negative individuals can be avoided. This given forms the basis for the advice in the ESPGHAN CD Guidelines to use HLA-typing as the first step of screening in risk-groups[2]. However, in FDRs the percentage of HLA-Q2/DQ8 negative individuals is low, in the cohorts we have studied for this thesis 12.5% (**Chapter 4**) and 15% (**Chapter 5**), quite comparable to what was found in other cohorts[38,39]. The same applies for diabetic patients, in whom several studies have demonstrated that absence of HLA-DQ2 and/or DQ8 haplotypes is scarce[40-42]. Together with the fact that HLA-typing is at present quite expensive and the difficulty for people to interpret the results, should prompt us to question this advice. On the other hand, in this day and age of shared decision making, it is not only up to the doctor to decide whether this absolute risk is something to know or not. We have demonstrated that parents of young children from coeliac families support HLA-typing and would repeat it in future children (**Chapter 4**). They would even be prepared to pay for the screening of their offspring[43]. In order to judge whether parents can actually be involved in such decisions, it is important not

only to know their opinion but also whether they are able to understand the background of genetics, which is notorious for its complexity and related cognitions. Despite the good knowledge that parents in coeliac families have with regard to HLA-typing on its own, misinterpretation of HLA negative results occurred in 48% of cases (**Chapter 4**). Parents who knew that presence of HLA-DQ2/DQ8 was necessary for individuals to be able to develop CD, thought that there was still a chance for their HLA-DQ2/DQ8 negative child to become a coeliac. Possibly, it is hard for them to adjust to a favourable outcome if the disease scenario disappears. It should however prompt physicians to make sure that parents understand the results and to improve the way of giving information. The information brochure that has been developed for this purpose in Dutch is attached as **Supplemental material appendix D** in this thesis. It can be helpful, especially since we found that parents who received favourable results were less inclined to look for additional information on HLA-genotyping and CD by themselves.

In addition, in the case of FDRs, HLA-typing can contribute to predict the individual risk to develop CD, which may have consequences for screening. Unfortunately, primary prevention by dietary interventions with breastfeeding and early or delayed introduction of gluten has proven not to be possible in at risk children[39,44]. In **Chapter 5**, we have presented the results of a retrospective analysis of CD screening in FDRs. We found a high prevalence of CD of 15%, even higher than earlier studies[45-47]. Several prospective studies have demonstrated the natural occurrence of CD in genetically at risk individuals[39,44,48], with a high prevalence ranging from 5 to 40% depending of the cohort, sex and genotype. Extrapolation of these data to older children and adults who are confronted with a family member being diagnosed with CD is however not straight forward. In this **Chapter 5**, we have shown that individual risk depends on the HLA-genotype, with HLA-DQ2 homozygosity resulting in the highest risk, therefor warranting closer surveillance. Our results suggest that the timing of CD specific antibody testing could be individualized depending on the relationship of the FDR with the index patient, the age of the FDR at time of the index diagnosis and HLA-type of the FDR. Prospective studies with regular screening intervals are needed to further address this issue, especially with regard to the adolescent age group. A proposal for a screening algorithm can be found in **Chapter 5** of this thesis (**Figure 3**).This would mean, although costly, that HLA-typing has its benefits even in this group, not only to rule out (future) CD, but mainly to estimate the risk of developing the disease.

Like FDRs, HLA-typing is also advised to be performed in children with T1DM as part of the coeliac screening process. Similar to FDRs, the vast majority of diabetic children is HLA-DQ2 and/or DQ8 positive (86%)[40]. About 7% of them have CD[40,49]. However, the diabetic children seem different than other risk groups, since there is a substantial group of children with T1DM who have fluctuating and/or normalizing CD specific antibodies[50-52]. On the other hand, like FDRs, older age at time of T1DM diagnosis has a

protective effect with regard to CD diagnosis[53]. In **Chapter 6**, we have shown the tendency in our cohort of diabetic children with CD as well to be younger than the diabetic children without CD. The usual female predominance of CD does not appear to be seen in other cohorts with T1DM and CD[53,54], even though in general there appears to be no gender difference in incidence of childhood T1DM[55]. Maybe the male preponderance is by part caused by a higher incidence of males in specific diabetic subgroups, like adolescents older than 13 years of age from European origin[55,56]. In our cohort however, we did not witness this male dominance, maybe due to relatively young age of our cohort (mean age 9.7 years).

In **Chapter 6**, we have demonstrated that when complying with the current ESPGHAN guidelines in asymptomatic children with T1DM, 12% of the children have normal duodenal mucosa when biopsied after ascertaining a TG2A titer of >3xULN. In accordance with our own results and other studies, repetition of serology instead of performing endoscopy to retrieve biopsies in these patients seems appropriate[50,51]. Current follow-up protocols for children with T1DM include CD specific serology at diagnosis and every 1-2 years thereafter[57]. In order to gather evidence on the length and interval of screening after diagnosis of T1DM prospective studies are needed. The international TEDDY (The Environmental Determinants of Diabetes in the Young) birth cohort study, studies factors influencing the development of T1DM, but also CD, because of the shared genetic background. It has been shown, that T1DM autoimmunity precedes coeliac autoimmunity in early childhood in children at high genetic risk of both diseases and that preceding islet autoantibodies (IA) significantly increase the risk of subsequent TG2A generation[58]. Data from the PreventCD cohort, shows a higher incidence of CD especially in multiple IA[59]. However, the time from IA seroconversion to clinical manifestation of T1DM shows a big variation between individuals, ranging from weeks to decades with individuals with different types of IAs having the highest risk in the shortest time[60], so CD can also precede T1DM. One can argue, that screening for IA could be done in coeliac children, since it was shown in the TEDDY cohort that genetically susceptible children who were diagnosed with T1DM diagnosed due to screening/surveillance have a better diabetes quality of life and lower parenting stress post-diagnosis compared to children diagnosed with T1DM in the community[61].

Future directions

In order to improve health-related quality of life of children with CD, it is important to find other ways to achieve early diagnosis and to optimize treatment and follow-up. In the next few years, special attention should be given to transition from pediatric to adult coeliac care. Ideally, this transition should be a collaborative process involving patients,

their parents or caregivers, the physician and the dietician[62]. Currently, the majority of coeliac patients in their twenties and forties who are diagnosed during childhood receive no medical or dietary supervision after transition to adulthood, with dietary non-compliance and complications such as iron deficiency anaemia and osteopenia as a result[63]. In 2016, the Prague consensus report on this matter was published[64], focussing on transfer of full responsibility for the adolescent, discussing dietary adherence and consequences of non-adherence and advising adult gastroenterologist on the approach for patients diagnosed during childhood based on the ESPGHAN[2] or NASPGHAN[3] criteria. Efforts should be made to endorse transition programs together with adult gastroenterologists. A better collaboration could also mean family programs by organising family outpatient clinics where within one family multiple FDRs could get their annual check-up whilst the others could be screened at the same time. Both knowledge on CD, self-management, family/risk group screening and transition could benefit from deploying medical applications and robots. Humanoid robots have been introduced in the health care domain for both adults[65,66] and children[67]. They could generate a continuous awareness of the chronicity of a disease whilst offering the support that is needed at any time and at any age. In this respect lessons will be learned from the PAL (Personal Assistant for healthy Lifestyle) 4U Project that started in 2015 as part of EU Horizon 2020 Program to improve child's diabetes regimen by assisting the child, health professional and parent. Another promising innovative example is the MyCyF-app, also funded by Horizon 2020, for patients with cystic fibrosis. Its goal is to enable them to monitor the disease, change enzyme-treatment and diet if needed and educate patients, caregivers and health professionals. Like these chronic illnesses, coeliac follow-up programs could also benefit from similar self-management programs. For example, online self-management systems can encourage patients to improve participation in their own health care by dealing with their symptoms, treatment and lifestyle changes. It can contribute to shared decision making between doctor and patient[68,69]. In CD, moving from traditional medical care with annual face-to-face 15-20 minutes visits focussing on complaints, growth and blood results to online consultations with questionnaires that also address quality of life and dietary adherence might be the way forward. Our research group has shown that implementation of eHealth is feasible for children with CD. It is cost saving, increases CD-specific health-related quality of life and is satisfactory in the majority of patients and parents[70]. Introducing robots or apps into coeliac care should incorporate several domains: 1. Education and information on the disease and treatment, not only for the patient and his/her family but also for use at school, restaurants etc, 2. Real-time diet evaluation, for example by using barcodes, with regard to gluten content and nutritional value, 3. Chat function with peers and/or professionals if needed (medical doctor, dietician, psychologist). Alliance with health science and technology together with the national coeliac association is needed in order to complement the needs of patients and to find the best eHealth solution.

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CHAPTER 8

Algemene discussie en conclusie

Alhoewel coeliakie (CD) een veel voorkomende, maar nog steeds onder gediagnosticeerde ziekte is, dient onderzoek zich niet alleen te focussen op de diagnostiek en nieuwe behandelmogelijkheden. Het zou zich ook moeten richten op de juiste begeleiding en monitoring van patiënten na starten van een glutenvrij dieet (GFD). Daarnaast zouden er in het kader van secundaire preventieve evidence-based richtlijnen voor risicogroepen dienen te komen om de ziekte zo vroeg mogelijk op te sporen. De vragen die bij aanvang van dit proefschrift voorlagen, worden gepresenteerd in **Tabel 1**, samen met de verzamelde aanbevelingen.

Tabel 1 *Voornaamste conclusies van dit proefschrift*

Vragen	Bevindingen	Aanbevelingen
Houden voedingstekorten aan of ontstaan deze bij kinderen met coeliakie na start van een glutenvrij dieet?	Voedingstekorten herstellen binnen 1 jaar op een glutenvrij dieet.	Na aan jaar zijn standaard bloedbepalingen naast coeliakie specifieke antistoffen zijn niet nodig.
Kunnen korte dieetvragenlijsten infrequente dieetfouten opsporen bij kinderen met coeliakie?	De korte dieetvragenlijst ontwikkeld door Biagi verschaft niet meer informatie dan coeliakie specifieke serologie.	Een gestandaardiseerd dieet-interview geeft gedetailleerde informatie over dieet(on)trouw, vooral als deze ingevuld wordt voor het polibezoek.
Wat is de impact van HLA-screening bij kinderen met risico op het ontwikkelen van coeliakie?	Ouders van jonge kinderen in gezinnen met coeliakie onderstrepen het belang van HLA-typering. Zij zouden dit bij toekomstige kinderen herhalen.	HLA-typering zou aangeboden moeten worden aan kinderen uit risicogezinnen, met daarbij behorende uitleg.
Wat is de beste screeningsmethode voor eerstegraadsfamilieleden van nieuw gediagnosticeerde coeliakie patiënten?	Eenmalige screening kan genoeg zijn in adolescente broers en zussen en in ouders van nieuw gediagnosticeerde coeliakie patiënten.	Screening door middel van coeliakie specifieke serologie zou met enige regelmaat moeten worden aangeboden aan alle HLA-DQ2 en/of DQ8 positieve eerstegraadsfamilieleden jonger dan 10 jaar.
Wanneer moeten duodenumbiopten gedaan worden bij kinderen met type1 diabetes en verhoogde TG2A serologie, gezien het feit dat serologie vaak vals positief is en/of een spontaan dalende trend laat zien bij deze kinderen?	12% van de asymptomatische kinderen met T1DM heft normale duodenum mucosa als zij gebiopteerd worden bij een TG2A titer van >3xULN.	Follow-up door middel van serologie in plaats van endoscopie voor het verkrijgen van biopten lijkt in deze patiënten veilig en verantwoord.

Discussie en aanbevelingen

Tot op heden is het glutenvrije dieet de enige behandeling van coeliakie. Alhoewel het een bewezen positief effect heeft op de gezondheid van coeliakie patiënten, ontbreken effectieve lange termijn behandelprogramma's voor zowel kinderen als volwassenen met coeliakie. De behoefte aan deze programma's om dieettrouw en uitkomsten te verbeteren wordt onderkend door verschillende experts[1-5], aangezien vertraging van het glutenvrij dieet lijkt te leiden tot een verhoogd risico op co-morbiditeit, mortaliteit en een lagere kwaliteit van leven[6,7]. In 2016 zijn daarom door kinderartsen-MDL in de Verenigde Staten aanbevelingen gepubliceerd voor de behandeling van kinderen met coeliakie[8], waarin het tekort aan gegevens van goede kwaliteit over dit onderwerp werd benadrukt. Momenteel bestaat standaard zorg voor kinderen met coeliakie uit regelmatige bezoeken aan een kinderarts(-MDL), met beoordeling van de algemene gezondheid, lengte en gewicht, dieettrouw en bloedtesten inclusief coeliakie specifieke antistoffen en andere bepalingen ter beoordeling van tekorten en co-morbiditeit. Het is daarbij belangrijk te realiseren dat eerdere publicaties hebben laten zien dat follow-up niet plaatsvindt bij alle patiënten, zowel bij kinderen als volwassenen[9-12]. Onderzoek in een pediatrisch cohort uit Israël heeft laten zien dat zogenaamde lost-to-follow-up patienten slecht gecontroleerde ziekte hadden met meer dieet ontrouw en positieve coeliakie specifieke antistoffen[13]. We kunnen alleen maar speculeren of deze non-compliance leidt tot meer lange termijn complicaties, aangezien follow-up gegevens gewoonweg ontbreken, bij kinderen en volwassenen, maar ook bij onbehandelde en behandelde patiënten. Het is daarom wezenlijk relevant evidence based richtlijnen te ontwikkelen voor follow-up van patiënten met coeliakie, die worden behandeld met een glutenvrij dieet.

Dit was de aanleiding de voedingstekorten te bestuderen die kunnen voorkomen in patiënten met coeliakie en die gewoonlijk ook gecontroleerd worden tijdens follow-up. De vaak bij diagnose aanwezige tekorten verdwijnen binnen een jaar op een glutenvrij dieet, zoals we hebben laten zien in **Hoofdstuk 2**. Dit betekent dat standard bloedtesten naast coeliakie specifieke antistoffen niet nodig zijn na een jaar follow-up. Dit is een belangrijke bevinding, vanwege de consequenties voor de organisatie van zorg voor kinderen met coeliakie, omdat bloedtesten tijd en geld kosten en door sommige kinderen als pijnlijk en stressvol worden ervaren. De gevonden tekorten in onze studie waren vergelijkbaar in aantal met eerdere studies, behalve vitamine B12 dat veel lager was in ons cohort (2%) in vergelijking met eerdere onderzoeken met adolescenten en volwassenen (12-41%)[14-17]. Onze gegevens over schildklierproblematiek (ongeveer 4%) zijn ook vergelijkbaar met eerdere studies, met een prevalentie van schildklier autoimmuniteit (verhoogd TSH of aanwezigheid van thyroperoxidase (TPO) antilichamen), hypothyroidie and hyperthyroidie variërend van 10-26%, 2-6% en 1%, respectievelijk[18,19]. De rationale achter het bepalen van de schildklierfunctie tijdens de follow-up zit in de

hoge frequentie van schildklier auto-immuniteit bij coeliakie[20], maar er is inconsistent bewijs over het beschermend effect van het glutenvrij dieet in het ontstaan ervan[21-23]. Op grond van onze resultaten, zou routinematig testen van TSH, de meest gebruikte bepaling om te screenen op schildklierziekten, ontmoedigd moeten worden, aangezien (tijdelijk) afwijkende resultaten vaak voorkomen zonder afwijkende FT4 waarden. Het kan leiden tot overdiagnose en angst bij patiënten en ouders. Schildkliertesten zouden alleen gedaan moeten worden bij symptomatische kinderen met een afwijkende groei of puberteitsontwikkeling, vermoeidheid, veranderd ontlastingspatroon en eetlust, spierpijn of tremoren, ofthalmopatie, thermodysregulatie en veranderde schoolprestaties. Indien nodig, zou FT4 bepaald dienen te worden. Omdat herstel van de mucosa na start van het glutenvrije dieet bij kinderen vergelijkbaar lijkt te zijn met volwassenen, is onze hypothese dat hetzelfde advies gegeven kan worden aan volwassenen, maar er is momenteel geen bewijs om dit te staven.

Om behandeling en follow-up bij kinderen te evalueren is een goede beoordeling van dieettrouw nodig, aangezien het dieet de enige behandeling is. Omdat het dieet niet altijd makkelijk is om te volgen, is het cruciaal de patiënten te identificeren die het dieet wel en niet volgen. Terwijl een uitgebreide beoordeling door een getrainde diëtiste beschouwd wordt als de beste manier om dieettrouw te evalueren, is voor deze methode veel tijd (20-30 minuten per patiënt) en gespecialiseerd personeel nodig. In **Hoofdstuk 3** hebben we laten zien dat een gestandaardiseerde dieetvragenlijst een goed alternatief kan zijn voor het lijfelijke contact met een diëtiste. Een korte vragenlijst, ontwikkeld en getest bij volwassenen[24] verschafte niet meer informatie over dieettrouw dan anti-tissue transglutaminase type 2 antistoffen (TG2A). Beiden detecteren niet alle fouten in kinderen en tieners met coeliakie. We hebben een verminderde dieetcompliance geconstateerd bij adolescenten, hetgeen eerder beschreven is in coeliakie populaties[25-27]. Geslacht, leeftijd bij diagnose en aanwezigheid van andere gezinsleden met coeliakie beïnvloedden de compliance niet, het volgen van een ander dieet naast het glutenvrije dieet en de aanwezigheid van klachten na gluteningestie evenmin. Ondanks pogingen hiertoe is het ons niet gelukt de vragenlijst in te korten. Door het toenemende gebruik van elektronische patiënten dossiers en eHealth tools zou de dieetvragenlijst echter toch ingezet kunnen worden in de zorg voor kinderen en jongvolwassenen met coeliakie. Routinematig gebruik van deze dieetvragenlijsten, vooral indien ingevuld voorafgaand aan een consult, kan de helpen te focussen op valkuilen en problemen met het glutenvrije dieet. Het kan de arts inzage geven in mogelijke dieetfouten, de redenen hiervoor en wanneer ze optreden. Dit biedt de mogelijkheid tot eventuele benodigde educatie en tot steun. Wij verwachten dat dit zal leiden tot verbetering van zorg voor patiënten met coeliakie, door hen op de korte termijn te versterken naar een betere dieettrouw, en mogelijk op de lange termijn complicaties van ziekte zal verminderen. Het zal niet alleen een nuttig instrument blijken in de dagelijkse praktijk, het dieetinterview kan ook

gebruikt in prospectieve studies naar lange termijn uitkomsten van coeliakie patiënten op een glutenvrij dieet. Nieuwe methoden om gluten immunogene peptiden (GIP) in urine en ontlasting te meten zouden hier verder aan bij kunnen dragen[28,29]. Door middel van het meten van GIP kan directe en kwantitatieve gluteninname beoordeeld worden. Het kan incidentele dieetfouten opsporen die niet opgepikt worden door de coeliakie specifieke serologie en daarmee patiënten identificeren die het dieet niet volgen. Omdat GIP analyse alleen gluten kan detecteren wanneer deze een paar dagen voorafgaand aan de test ingenomen is, kan ingestie hiervoor gemist worden. GIP bepaling kan nuttig zijn voor patiënten die het dieet goed volgen, maar bij wie het TG2 antistoffen positief blijven. Als de TG2A-waarde daalt en GIP is bij herhaling negatief, lijkt geruststelling van patiënten en ouders op zijn plaats.

De volgende stap in het verbeteren van de gezondheid en kwaliteit van leven voor coeliakie patiënten is het verminderen van het aantal gemiste diagnoses. Om dit mogelijk te maken is notie van de ziekte en eerder denken eraan belangrijk, zowel bij het publiek als bij artsen. Daarbij dient secundaire preventie door vroege diagnose en behandeling geoptimaliseerd te worden door de ontwikkeling van screeningsprogramma's voor risisogroepen. Betreffende screening zijn de voordelen die het oplevert het meest in het oog springend. Voorgaand onderzoek laat enig bewijs zien voor screening strategieën ter voorkoming van complicaties en reducering van medische kosten[30-32]. Echter, voordelen en kosteneffectiviteit van screening blijven controversieel[33,34.] Actieve opsporing van patiënten (case finding) kan overwogen worden, alhoewel het algemeen bekend is dat het gebruik van symptomen om patiënten te identificeren slechts beperkte waarde heeft. Coeliakie gerelateerde klachten komen zowel bij individuen met en zonder coeliakie voor[35]. Aan de andere kant zouden case finding programma's gebaseerd op symptomen bij kinderen een alternatief kunnen zijn voor bevolkingsonderzoek, waar de Medisch Ethische Toetsing Commissies in Nederland tegen gekant zijn. Omdat gezondheidsvoordelen bij symptomatische kinderen na diagnose en behandeling te verwachten zijn, werd toestemming verleend aan de GLUTENSCREEN studie binnen de jeugdgezondheidszorg in Noord-Holland. Bij risicogroepen wordt screening op coeliakie aanbevolen door zowel de Nederlandse als Europese coeliakie richtlijnen[2,36], aangezien mensen met andere auto-immuunziekten zoals type 1 diabetes mellitus (T1DM), auto-immuun schildklier- en leverziekten, maar ook syndromen, zoals Down, Turner en Williams syndroom, selectieve IgA deficiëntie en eerstegraadsfamilieleden van coeliakie patiënten een hoger risico hebben de ziekte te krijgen. Betrokkenheid van huisartsen in Nederland hierbij is hoognodig, vanwege het feit dat de NHG standaard screening bij eerstegraadsfamilieleden, die meestal alleen bij de huisarts onder controle zijn, niet adviseert[37].

Door de hoge negatief voorspellende waarde van HLA-typering, kunnen onnodige onderzoeken achterwege blijven bij HLA-DQ2 en DQ8 negatieve mensen. Dit gegeven vormt de basis van het advies in de ESPGHAN richtlijn coeliakie om HLA-typering te gebruiken als eerste stap in de screening van risicogroepen2. Het percentage van HLA-DQ2/DQ8 negatieve eerstegraadsfamilieleden is alleen laag, in de cohorten die we in dit proefschrift hebben bestudeerd 12.5% **(Hoofdstuk 4**) en 15% (**Hoofdstuk 5**), vergelijkbaar met andere cohorten[38,39]. Hetzelfde geldt voor patiënten met diabetes, bij wie in verscheidene studies is aangetoond dat de HLA-DQ2 en/of DQ8 haplotypes slechts zelden afwezig zijn[40–42]. Het feit dat HLA-typering nog steeds vrij prijzig is en de uitslag bovendien voor veel mensen lastig te interpreteren is, zou ons dit advies in twijfel moeten laten trekken. Aan de andere kant, in deze tijd van shared decision making, is het wel of niet weten van het risico niet aan de arts om te bepalen. We hebben laten zien dat ouders uit gezinnen waarin coeliakie voorkomt achter HLA-typering staan en dit bij toekomstig kinderen zouden herhalen (**Hoofdstuk 4**). Ze zouden zelfs bereid zijn te betalen voor het screenen van hun nakomelingen[43]. Om te beoordelen of ouders goed in staat zijn bij zulke beslissingen betrokken te worden, is het belangrijk om naast hun mening ook zicht te hebben op hun begrip van erfelijkheid, wat berucht staat om de complexiteit en de erbij behorende opinies. Ondanks de goede kennis van ouders uit gezinnen met coeliakie ten aanzien van HLA-typering, mis interpreteert 48% van hen een negatieve uitslag (**Hoofdstuk 4**). Ouders die wisten dat HLA-DQ2/DQ8 nodig is voor de ontwikkeling van coeliakie dachten dat er toch een kans was dat hun HLA-DQ2/DQ8 negatieve kind coeliakie zou kunnen krijgen. Misschien is het moeilijk voor hen om aan de gunstige uitslag te wennen met het verdwijnen van het ziekte perspectief in de toekomst. Het zou artsen moeten laten achterhalen of ouders de uitslag begrijpen en de manier van informatievoorziening laten verbeteren. De informatiebrochure die voor dit doel is ontworpen, is als **Supplemental material appendix D** toegevoegd aan dit proefschrift. Het kan helpen, juist bij ouders, die zoals wij zagen, bij een gunstige uitslag niet meer geneigd waren extra informatie op te zoeken over HLA-typering en coeliakie. Daarbij kan HLA-typering bij eerstegraadsfamilieleden helpen het risico in te schatten op het ontwikkelen van coeliakie, hetgeen consequenties kan hebben voor de screening. Helaas is primaire preventie door middel van dieet interventies met vroege en verlate introductie van gluten niet mogelijk gebleken[39,44]. In hoofdstuk 5 hebben de resultaten gepubliceerd van een retrospectieve analyse van coeliakie screening in eerstegraadsfamilieleden. We ontdekten een hoge prevalentie van coeliakie van 15%, zelfs hoger dan eerdere studies[45–47]. Verschillende prospectieve studies hebben laten zien hoe vaak coeliakie in erfelijk belaste individuen voorkomt[39,44,48], met een hoge prevalentie, variërend van 5 tot 40% afhankelijk van het cohort, geslacht en HLA-genotype.

Extrapolatie van deze data naar oudere kinderen en ouders, die geconfronteerd worden met een gezinslid met nieuw ontdekte coeliakie, is echter niet zo eenvoudig. In dit hoofdstuk 5 hebben we laten zien dat het individuele risico afhangt van HLA-genotype, met het hoogste risico bij HLA-DQ2 homozygotie, wat tot striktere controle zou moeten leiden. Onze resultaten suggereren dat coeliakie specifieke antistoftesten individueel gepland zouden kunnen worden, afhankelijk van de relatie van het eerstegraadsfamilielid met de index patiënt, de leeftijd van het familielid ten tijde van de diagnose van de index patiënt en het HLA-type van het familielid. Prospectieve studies met geplande screeningsintervallen zijn nodig om dit punt verder te onderzoeken, vooral ten aanzien van de adolescenten. Een voorstel voor een screeningsalgoritme kan teruggevonden worden in hoofdstuk 5 van dit proefschrift (**Figuur 3**). Dit kan betekenen, dat ondanks de hoge kosten, HLA-typering zijn voordelen heeft in deze groep, niet alleen om coeliakie (in de toekomst) uit te sluiten, maar vooral om het risico erop in te schatten.

Net als bij eerstegraadsfamilieleden wordt HLA-typering aangeraden bij kinderen met type 1 diabetes mellitus als onderdeel van het screeningsproces op coeliakie. Vergelijkbaar is het feit dat de meerderheid van de kinderen met diabetes HLA-DQ2 en/of DQ8 positief is (86%)[40]. Ongeveer 7% van hen heeft coeliakie[40,49]. Daarentegen lijken de kinderen met diabetes ter verschillen van de andere risicogroepen, aangezien een substantiële groep fluctuerende en/of normaliserende coeliakie specifieke antistoffen heeft[50-52]. Aan de andere kant, net als bij de eerstegraadsfamilieleden beschermt oudere leeftijd ten tijde van ontwikkelen van diabetes ten aanzien van coeliakie diagnose[53]. In **Hoofdstuk 6**, zagen we dezelfde trend van een jongere leeftijd van diabetes kinderen met coeliakie ten opzichte van kinderen met diabetes zonder coeliakie. De gebruikelijke vrouwelijke overheersing zoals die gezien wordt bij coeliakie lijkt niet te bestaan bij eerder beschreven cohorten met type 1 diabetes en coeliakie[53,54], alhoewel er in zijn algemeenheid geen verschil lijkt te zijn betreffende incidentie van diabetes op de kinderleeftijd qua geslacht[55]. Wellicht dat de mannelijke dominantie te verklaren valt door de hogere incidentie van jongens in specifieke diabetes subgroepen, zoals adolescenten ouder dan 13 jaar van Europese komaf[55,56]. In ons cohort, wordt de mannelijke overheersing echter niet gezien, misschien door de relatieve jonge leeftijd van onze groep (mean leeftijd 9.7 jaar). In **Hoofdstuk 6**, hebben we laten zien dat 12% van de asymptomatische kinderen met diabetes type 1 hebben normale duodenum mucosa, wanneer zijn conform de huidige ESPGHAN richtlijn gebiopteerd worden bij een TG2-titer van >3x de bovengrens van normaal. In overeenstemming met onze resultaten en andere studies lijkt herhaling van serologie in plaats van endoscopie ter verkrijging van biopten doelmatig[50,51]. Conform de huidige behandelprotocollen voor kinderen met type 1 diabetes wordt coeliakie specifieke serologie bij diagnose bepaald en nadien iedere 1-2 jaar[57].

Prospectieve studies zijn nodig om bewijs te vergaren ten aanzien van de screeningsduur na de diabetes diagnose en het interval. De internationale TEDDY geboortecohort studie (The Environmental Determinants of Diabetes in the Young) bestudeert factoren die de ontwikkeling van type 1 diabetes, maar ook coeliakie kunnen beïnvloeden, gezien de gemeenschappelijke erfelijke achtergrond. Hierbij werd getoond dat diabetes autoimmuniteit vooruit loopt op coeliakie auto-immuniteit gedurende de jeugd van kinderen met een erfelijke aanleg voor beide ziekten, waarbij vorming van zo genaamde "islet antibodies" (IA) de kans op het maken van TG2 antistoffen significant vergroot[58]. Gegevens uit het PreventCD cohort laten een hogere incidentie van coeliakie zien in geval van meerdere subtypen IA[59]. Aangezien de tijd tussen IA seroconversie tot klinische manifestatie van T1DM erg kan verschillen van individu tot individu, variërend van weken tot decennia[60], kan coeliakie echter diabetes vooraf gaan. Het valt te bezien of screening met IA gedaan dient te worden in kinderen met coeliakie, omdat erfelijk belaste kinderen bij wie diabetes vastgesteld werd door middel van screening een betere kwaliteit van leven hebben en hun ouders minder stress hebben na de diagnose vergeleken bij kinderen met diabetes uit de algehele bevolking[61].

Toekomstvisie

Om gezondheid gerelateerde kwaliteit van leven van kinderen met coeliakie te verbeteren, is het belangrijk manieren te vinden ter bevordering van een vroege diagnose en voor optimalisering van behandeling en controle. De aankomende jaren dient speciale aandacht te gaan naar transitie van zorg van kind naar volwassen coeliakie zorg. Idealiter zou deze transitie een gezamenlijk proces moeten zijn met patiënten, hun ouders of verzorgers, artsen en dietisten[62]. Momenteel ontvangt de meerderheid van de patiënten met coeliakie tussen 20 en 40 jarige leeftijd geen medische of dietetaire zorg na transitie naar volwassenheid, met dieet-ontrouw en complicaties zoals als ijzertekort anemie en osteopenie als resultaat[63]. In 2016, werd het zogenaamde Praag consensus rapport ten aanzien van dit onderwerp gepubliceerd[64], met focus op het verschuiven van volledige verantwoordelijkheid naar de adolescent zelf, aandacht voor dieet trouw en consequenties van dieet ontrouw en advies aan MDL-artsen ten aanzien van de aanpak van coeliakie patiënten die in hun kindertijd conform ESPGHAN[2] of NASPGHAN[3] criteria zijn gediagnosticeerd. Er zal gepoogd moeten worden transitie programma's samen met MDL-artsen te ondersteunen. Een betere samenwerking zou kunnen resulteren in maatwerk voor gezinnen, met gezinsconsulten waarin een of meerdere gezinsleden met coeliakie hun jaarlijkse controle kunnen hebben terwijl de anderen tegelijkertijd gescreend kunnen worden op de ziekte.

Zowel kennis over coeliakie, zelfmanagement, familie en risicogroep screening en transitie zou gebaat kunnen zijn bij de inzet van medische applicaties en robots. Menselijke robots zijn in het gezondheidsdomein zowel bij volwassenen[65,66] als bij kinderen[67] geïntroduceerd. Zij kunnen de continue aanwezigheid van een chronische ziekte onder de aandacht brengen en gelijktijdig ondersteuning bieden waar en wanneer dat nodig is, op elke leeftijd. Hierbij zou lering getrokken kunnen worden uit het PAL (Personal Assistant for healthy Lifestyle) 4U Project dat in 2015 is gestart als onderdeel van het EU Horizon 2020 Programma dat als doel heeft de zorg voor kinderen met diabetes te verbeteren door het kind, de ouder en de gezondheidsprofessional bij te staan. Een ander veelbelovend en innovatief voorbeeld is de MyCyF-app, ook gefinancierd uit Horizon 2020, voor patiënten met taaislijmziekte (cystic fibrosis). Met als doel om ziekte te monitoren, enzymtherapie en dieet waar nodig aan te passen en patiënten, verzorgers en professionals van educatie te voorzien. Net als deze chronische ziekten zouden coeliakie programma's baat kunnen hebben bij vergelijkbare zelfmanagement programma's. Online zelfmanagement programma's zouden patiënten kunnen laten participeren in hun eigen gezondheidszorg door om te gaan met hun klachten, behandeling en leefstijlinterventies. Het kan bijdrage aan zogenaamd "shared decision making" tussen patiënt en arts[68,69]. De overgang van ouderwetse medische zorg met jaarlijkse "face-to-face" contacten van 15-20 minuten met de focus op klachten, groei en bloeduitslagen naar online consulten met vragenlijsten over kwaliteit van leven en dieettrouw kan een sprong voorwaarts worden. Onze onderzoeksgroep heeft laten zien dat implementatie van eHealth is haalbaar bij kinderen met coeliakie. Het bespaart kosten, vergroot coeliakie specifieke gezondheid gerelateerde kwaliteit van leven en stelt de meerderheid van de patiënten tevreden[70]. De introductie van robots en apps in de coeliakie zorg zou meerdere domeinen moeten behelzen: 1. Educatie en informatie over de ziekte en behandeling, niet alleen voor de patiënt en zijn/haar gezin, maar ook voor gebruik buitenshuis op school, in restaurants etc., 2. Realtime dieetbeoordeling, bijvoorbeeld door het scannen van barcodes, met betrekking tot gluten gehalte en voedingswaarde, 3. Chat functie met leeftijdsgenoten en of professionals indien nodig (arts, diëtiste, psycholoog). Samenwerking tussen gezondheidszorg, technologische partners en de Nederlandse Coeliakie Vereniging is nodig om de behoeften van patiënten aan te vullen en de beste eHealth oplossing te vinden.

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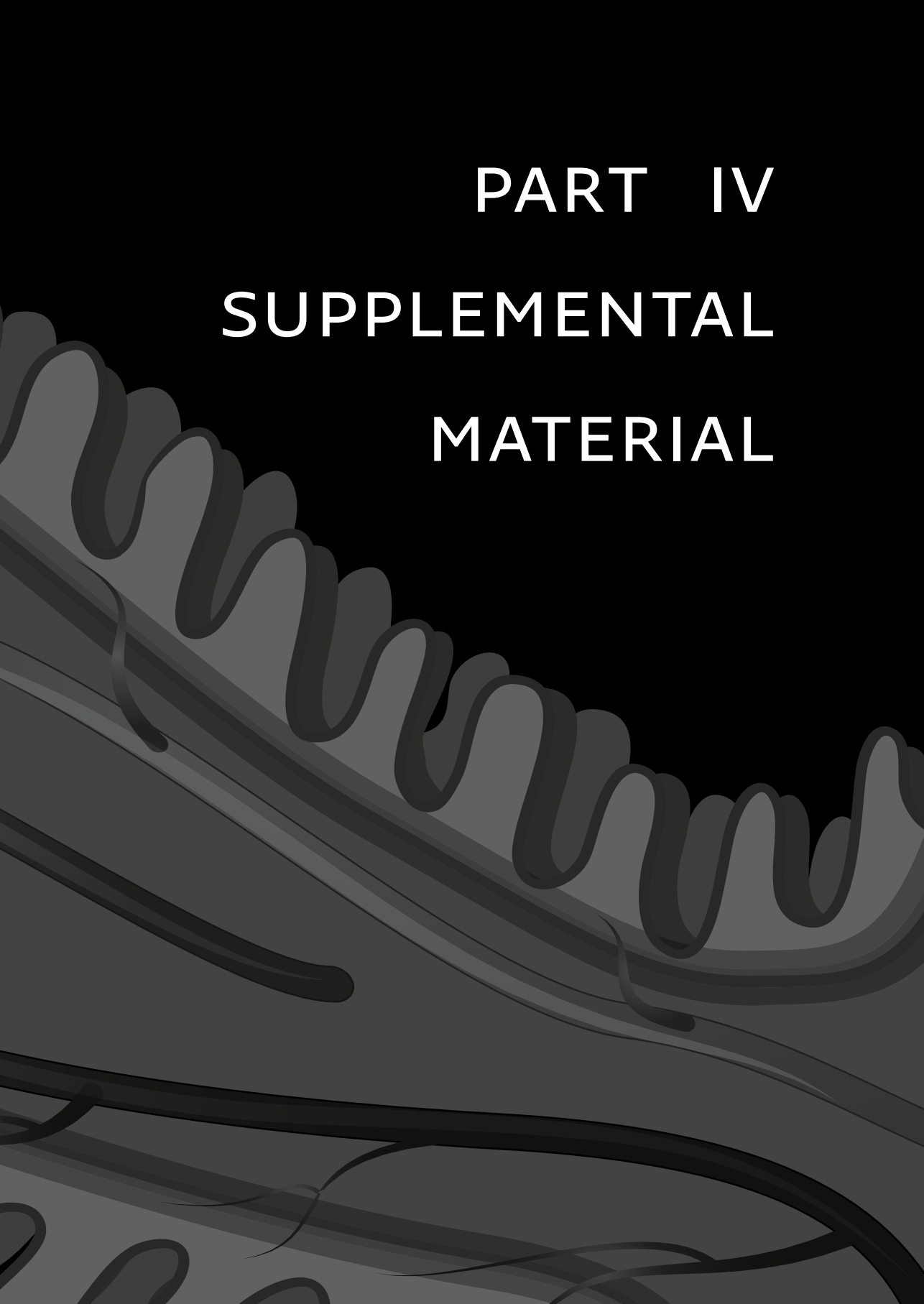
PART IV

SUPPLEMENTAL MATERIAL



A. LIST OF ABBREVIATIONS

CD Coeliac disease
TG2A Anti-tissue transglutaminase type 2 antibodies
HLA Human leucocyte antigen
T1DM Type 1 diabetes mellitus
EMA Anti-endomysium antibodies
DGPA Deamidated gliadin peptide antibodies
ESPGHAN European Society for Paediatric Gastroenterology, Hepatology and Nutrition
GFD Gluten-free diet
FDRs First degree relatives
ULN Upper limit of normal
ID Iron deficiency
IDA Iron deficiency anemia
NIH National Institutes of Health
NICE National Institute for Health and Care Excellence
NASPGHAN North American Society for Paediatric Gastroenterology, Hepatology and Nutrition
LUMC Leiden University Medical Center
FT4 Free thyroxin
TSH Thyroid stimulating hormone
AbTPO Thyroperoxidase antibodies
DQ+ HLA-DQ2 and/or DQ8 positive
DQ- HLA-DQ2 and/or DQ8 negative
HRQoL Health related quality of life
HADS Hospital Anxiety and Depression Scale
TAPQOL TNO-AZL preschool children quality of life questionnaire
ROC Receiver operating characteristics
CoN Cut-off values for normality
GIP Gluten immunogenic peptides

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1 *Dietary interview, written version.*

- Between brackets number of points per answer, leading to scores:
- 0-2 strict gluten-free diet
- 3-20 gluten-free diet with important errors
- 21-84 gluten-free diet not followed

1 Is your child on a gluten-free diet (GFD)? Yes(84)/No(0)

If no, why isn't your child on a GFD? (tick appropriate answers)
- Because my child doesn't have coeliac related complaints
- Because it is too complicated
- Because it is too expensive
- Some other reason, being ...

Questionnaire ends here if answer was no.

2 Does your child eat gluten accidentally? Yes/No

If yes: (tick appropriate answer)
- Every day (2)
- Once a week (1)
- Once a month (0)
- Once a year (0)

3 Does your child eat gluten intentionally? Yes/No

If yes, this consists of: (tick appropriate answer)
- Bread/cereals: daily (5), weekly (4), monthly (2), once a year (0)
- Pastry: daily (5), weekly (4), monthly (2), once a year (0)
- Pizza: daily (5), weekly (4), monthly (2), once a year (0)
- Oliebollen (traditional Dutch deep-fried solid doughnuts eaten during New Year's Eve and fun fairs): daily (5), weekly (4), monthly (2), once a year (0)
- Deep-fried snacks: daily (3), weekly (2), monthly (1), once a year (0)
- Candy bars, candy, crisps, nuts: daily (2), weekly (1), monthly (0), once a year (0)
- Other food such as ... daily (3), weekly (2), monthly (1), once a year

If yes, my child eats gluten intentionally: (tick appropriate answers)
- At home
- When he/she is with other family (like grant parents)
- When he/she is with friends
- At special occasions (birthday, party etc)
- When eating out
- At school/work
- During sport activities
- During camp
- During holidays

4 If there is a treat at school or at work, my child will eat the treat even if it might contain gluten. Yes(2)/No(0).

5 We'll discuss the GFD with the person taking care of the meal provided for my child, if my child will eat somewhere else.Yes/No.

If no, we do not do so: (tick appropriate answers)
- On holiday (3)
- On a school trip (3)
- On camp (3)
- When staying overnight (2)
- At a party (1)

6 At home, my child is the only one on a GFD. Yes/No.

If no, who else is on a GFD?
- Father
- Mother
- Sibling

7 Besides gluten-free products, there are also gluten containing products available at our home. Yes/No.

If yes:
- The gluten containing products are stored separately from the gluten-free products. Yes(0)/No(2).
- Other people, who are not on a GFD, can use the gluten-free butter and spreadable sandwich toppings. Yes/No.
- If yes, is this done with clean utensils? Yes(0)/No(2).
- If gluten-free and gluten containing flour is used, the gluten-free flour is always used first. Yes(0)/No(2).

8 Gluten-free food is always prepared with clean hands, worktop and materials. Yes(0)/No(2).

9 When needed, gluten-free food is prepared with a personal toaster, bread box, deep fryer or baking tin. Yes(0)/No(2).

10 My child eats gluten-free bread. Yes/No.

If yes, this bread is:
- Home baked, using flour with a 'gluten-free' label or logo. Yes/No.
- Bought as prepacked bread. Yes(0)/No(5).
- Bought at a local bakery, who makes the bread by itself. Yes(2)/No(0).

If no, why not:
- My child does not eat bread. Yes/No.
- My child eats gluten containing bread. Yes(5)/No(0).

11 My child only eats pasta products with a gluten-free label or logo on the packaging. Yes(0)/No(3).

12 My child only eats pastries and cereals with a gluten-free label or logo on the packaging. Yes(0)/No(3).

13 My child eats naturally gluten-free flour (like corn, rice, buckwheat, oats, quinoa, teff). Yes/No.

If yes, only with a gluten-free label or logo on the packaging. Yes(0)/No(3).

14 My child eats food containing wheat starch. Yes/No.

If yes: (tick appropriate answer)
- Every day (2)
- Once a week (1)
- Once a month (0)
- Once a year (0)

15 My child eats food containing gluten-free wheat starch. Yes/No.

16 My child eats food with a label "may contain traces of gluten or wheat". Yes/No.

If yes: (tick appropriate answer)
- Every day (2)
- Once a week (1)
- Once a month (0)
- Once a year (0)

17 My child eats food with a label "prepared in an environment where gluten/wheat is processed". Yes/No.

If yes: (tick appropriate answer)
- Every day (2)
- Once a week (1)
- Once a month (0)
- Once a year (0)

18 My child drinks gluten containing beer. Yes/No. (only presented to children > 12 years)

If yes: (tick appropriate answer)
- Every day (2)
- Once a week (1)
- Once a month (0)
- Once a year (0)

19 If my child has to use medication, we make sure it is gluten-free. Yes(0)/No(1).

20 If we do not know that certain food is gluten-free:
- We'll check whether it has a gluten-free label or logo. Yes/no.
- We'll check whether it has a gluten-free label from the supermarket. Yes/no.
- We'll read the ingredients and decide whether it is gluten-free. Yes/no.
- We'll cross-check is with the Livaad list (List of gluten-free food, provided by the Dutch Coeliac Society). Yes/no.
- We'll ask the manufacturer. Yes/no.
- My child will eat it and we will observe whether he/she get complaints. Yes/no.

21 If my child eats something with gluten, he/she gets complaints. Yes/no.

If yes: (tick appropriate answers)
- Abdominal pain. Yes/no.
- Diarrhea. Yes/no.
- Vomiting. Yes/no.
- Fatigue. Yes/no.
- Loss of appetite. Yes/no.
- Something else …

22 We believe that we have sufficient knowledge on the GFD. Yes/no.

23 I am worried whether my child's diet contains enough nutrients (like proteins, fat and vitamins). Yes/no.

24 It is important for me to have contact with a dietician about my child's diet on a regular basis. Yes/no.

If yes: (tick appropriate answer)
- Once a year
- Once every 2 years
- Once every 5 years
- Other time interval: …
- I would like to discuss: …

25 It is important for me to have contact with a doctor about my child's diet on a regular basis. Yes/no.

If yes: (tick appropriate answer)
- Once a year
- Once every 2 years
- Once every 5 years
- Other time interval: …
- I would like to discuss: …

26 My child is on another diet besides the GFD. Yes/No.

If yes: (tick appropriate answer)
- Lactose free
- Cow's milk free
- Other …

2 *Short dietary questionnaire*

Between brackets number of points per answer, leading to scores:
- 0-2 strict gluten-free diet
- 3-14 gluten-free diet with important errors
- 15 gluten-free diet not followed

1 Is your child on a gluten-free diet (GFD)? Yes(15)/No(0)

Questionnaire ends here if answer was no.

2 Does your child eat gluten intentionally? Yes(15)/No(0)

If yes, my child eats gluten intentionally: (tick appropriate answers)
- At home
- When he/she is with other family (like grant parents)
- When he/she is with friends
- At special occasions (birthday, party etc)
- When eating out
- At school/work
- During sport activities
- During camp
- During holidays

3 We'll discuss the GFD with the person taking care of the meal provided for my child, if my child will eat somewhere else. Yes(2)/No(0).

4 The gluten containing products are stored separately from the gluten-free products. Yes(0)/No(2).

5 Other people, who are not on a GFD, can use the gluten-free butter and spreadable sandwich toppings. Yes(2)/No(0).

6 When needed, gluten-free food is prepared with a personal toaster, bread box, deep fryer, baking tin. Yes(0)/No(2).

7 My child eats gluten-free bread bought at a local bakery, who makes the bread by itself. Yes(2)/No(0).

8 My child eats food containing wheat starch. Yes(2)/No(0).

9 My child eats food with a label “may contain traces of gluten or wheat”. Yes (2)/No(0).

10 My child eats food with a label “prepared in an environment where gluten/wheat is processed”. Yes(2)/No(0).

11 If my child has to use medication, we make sure it is gluten-free. Yes(0)/No(2).

D. HLA-INFORMATION FOLDER FOR CHILDREN WITH COELIAC DISEASE AND THEIR PARENTS

TEKST

SL Vriezinga
MMS Wessels

ILLUSTRATIES

SL Vriezinga

HLA-typering bij coeliakie

Bij uw kind en/of bij u wordt onderzoek gedaan naar coeliakie of is coeliakie vastgesteld. Vaak maakt HLA-typering deel uit van dit onderzoek. Deze folder is gemaakt voor patiënten en hun ouders, die geïnteresseerd zijn in de achtergrond van dit onderzoek. Een aantal termen wordt uitgelegd en de betekenis van de uitslag wordt besproken.

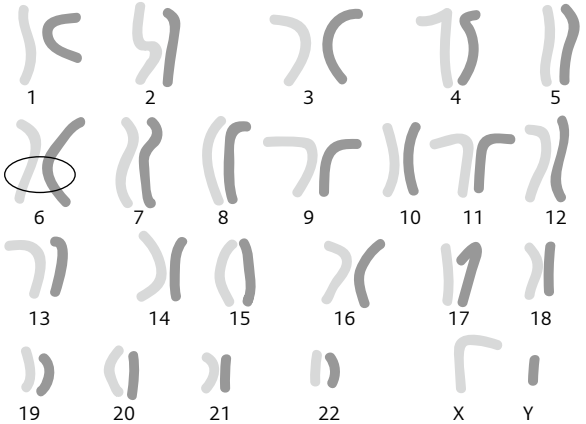
Genetica

Coeliakie is de meest voorkomende voedselintolerantie in de Westerse wereld; meer dan 1 op de 100 kinderen heeft coeliakie. Erfelijke factoren spelen een belangrijke rol bij de ontwikkeling van deze ziekte. Voordat we dieper in gaan op het onderwerp HLA-typering bij coeliakie, wordt eerst de achtergrond van de erfelijkheidsleer, de *genetica* besproken

1 Basis — Wat is genetica eigenlijk?
Genetica is een gebied in de natuurwetenschappen. Onderzoekers in de genetica houden zich bezig met de overerving van erfelijke eigenschappen van de ene generatie op de volgende. Hoe een organisme er uit ziet, zich gedraagt en hoe het zich voortplant, wordt bepaald door zijn/haar erfelijk materiaal. Het erfelijk materiaal is opgebouwd uit genen. Dit zijn een soort bouwsteentjes die samen het DNA vormen. In je DNA (en dus in je genen) zitten codes 'verborgen'. Deze codes geven instructies voor bijvoorbeeld bloedgroep, haarkleur, maar ook voor aanleg voor bepaalde ziekten.

DNA is opgebouwd uit genen. Een gen codeert voor een bepaalde eigenschap. Van elk gen hebben we één variant van onze moeder en één variant van onze vader. De genen liggen als compleet erfelijk materiaal opgeslagen in vrijwel iedere cel van ons lichaam.

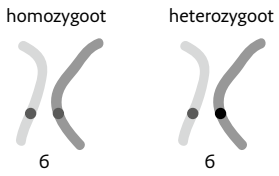
Figuur 1 *Schematische weergave van een karyotype (=chromosomenpatroon) van een man (XY; vrouwen hebben XX). Bedenk dat steeds de ene helft van het chromosoompaar van de vader afkomstig is, en de andere helft van de moeder. Op chromosoom 6 (cirkel) liggen de coeliakiegenen.*



Natuurlijk bestaan er uitzonderingen op die regel. Rode bloedcellen bijvoorbeeld, hebben geen kern en dus ook geen DNA. Een andere uitzondering wordt gevormd door ei- en zaadcellen. Zij hebben van elk gen maar één variant (in plaats van twee). Bij de bevruchting van de eicel door een zaadcel smelten de genen weer tot een set van twee varianten samen. Het totale DNA en dus het totale genen pakket, is verdeeld over 46 *chromosomen*, waarvan er 23 uit vaderlijk DNA en 23 uit moederlijk DNA bestaan. De genen die coderen voor een bepaalde eigenschap liggen bij ieder mens op precies dezelfde plaats op een chromosomenpaar (zie **Figuur 1**).

De inhoud van de informatie op beide chromosomen van een paar kan echter wel verschillend zijn. Zoals gezegd, hebben we van elk gen twee varianten; ook wel twee *allelen* genoemd. Een allel is een variant van een eigenschap. De 2 aanwezige allelen van een eigenschap kunnen gelijk zijn,bijvoorbeeld voor blauwe of voor bruine ogen (dit noemen we *homozygoot*, zie **Figuur 2**). Ze kunnen ook verschillen, bijvoorbeeld een allel voor blauwe ogen en een allel voor bruine ogen (dit noemen we *heterozygoot*). Als de allelen identiek zijn, is de uitkomst duidelijk, als de twee allelen coderen voor een verschillende oogkleur, zal de uiteindelijke oogkleur van het individu bepaald worden door het sterkste, dominante allel (bruin).

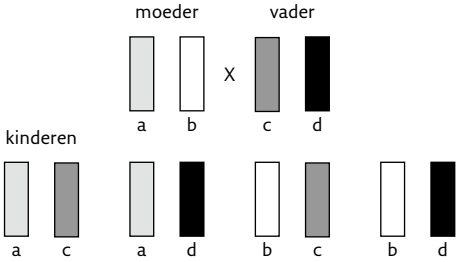
Figuur 2 *Twee maal een schematische weergave van chromosoompaar 6. De stippen geven de locatie van een gen op een chromosoom aan. De allelen op het linkerchromosoom zijn identiek; het individu is homozygoot voor de eigenschap (bijvoorbeeld 2x blauwe ogen). Rechts zijn de genen verschillend; deze persoon is heterozygoot voor de eigenschap (bijvoorbeeld blauwe ogen + bruine ogen).*



Overerving van een genetische eigenschap

De overerving van een genetische eigenschap is in **Figuur 3** schematisch weergegeven met een voorbeeld. Elk van beide ouders heeft in dit geval twee verschillende varianten van een eigenschap. De varianten noemen we *a* en *b*, *c* en 9. Een kind erft van elke ouder één chromosoom (iedere ei- en zaadcellen heeft slechts 23 chromosomen). Hij/zij erft dus van beide ouders één variant . Uiteindelijk kunnen er vier verschillende nieuwe combinaties ontstaan. De kans dat vervolgens een kind geboren wordt met dezelfde combinatie als zijn oudere broer of zus, is 25%. Als de moeder twee maal variant *a* heeft, zal elk kind in ieder geval ook variant *a* hebben, plus een variant van hun vader. Als beide ouders twee maal variant *c* hebben, zullen de kinderen ook twee maal variant *c* hebben.

Figuur 3 *Een schematische weergave van genen die coderen voor een willekeurige eigenschap. Beide ouders hebben twee unieke varianten van de eigenschap. Bij voortplanting tussen deze man en vrouw is de kans op elk van de vier nieuwe combinaties telkens 25%.*



Coeliakie & HLA-moleculen

Zoals reeds beschreven in tekstbox 1, kunnen onze genen ook coderen voor de vatbaarheid voor bepaalde ziekten. Het is onderzoekers opgevallen dat coeliakie vaak voorkomt in bepaalde families en in bepaalde bevolkingsgroepen. Zo werd ontdekt, dat het HLA-gen een belangrijke rol speelt in de ontwikkeling van coeliakie. HLA staat voor Humane (*menselijke*) Leukocyten (*witte bloedcellen*) Antigenen (*karakteristieken*). HLA-genen zijn nodig voor de opbouw van ons afweersysteem. Ze zijn onder te verdelen in klasse I en klasse II.

Coeliakie is geassocieerd met de HLA-klasse-II genen. Deze genen coderen voor zogenaamde HLA-II-moleculen. Deze moleculen worden ingebouwd op het oppervlak van bepaalde witte bloedcellen, *antigeen presenterende cellen* geheten. Ze spelen een belangrijke rol in ons afweersysteem, omdat ze onderscheid kunnen maken tussen alles wat lichaamseigen (*veilig*) en lichaamsvreemd (*potentieel bedreigend*) is. Als zij lichaamsvreemd materiaal gevonden hebben, zullen zij dit 'verklikken'. Dit doen ze door de lichaamsvreemde eiwitmoleculen (maar ook virussen en bacteriën, omdat zij voor een deel uit eiwitten bestaan), te binden aan een HLA-II-molecuul op hun celoppervlak. Vervolgens presenteren ze de eiwitten aan een andere 'afdeling' van het afweersysteem; de *T-cellen* (CD4+ T-helper cellen). Deze T-cellen kunnen onderscheid maken tussen daadwerkelijk bedreigende vreemde eiwitten en eigenlijk niet bedreigende vreemde eiwitten, zie tekstbox 2. Ons voedsel bijvoorbeeld, bestaat uit een scala aan lichaamsvreemd materiaal, waarvan het gros niet bedreigend voor ons is maar zelfs gezond! In de eerste levensjaren ontwikkelt een kind tolerantie voor zulke lichaamsvreemde, maar niet bedreigende eiwitten. Tijdens dit proces wordt de T-cellen aangeleerd deze eiwitten niet te zien als bedreigend. Want als dit wel gebeurt, start een keten van reacties, met als einddoel de vernietiging van het betreffende eiwit. Deze afweerreactie is dus

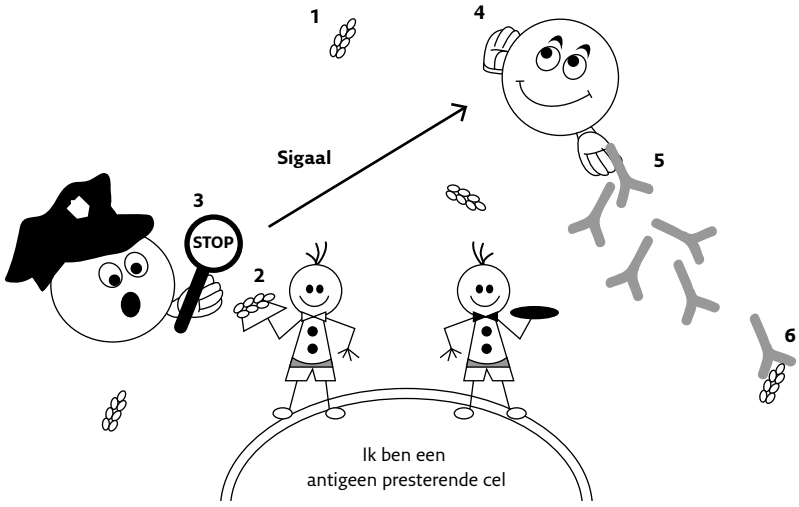
bedoeld om het lichaam te beschermen. Bij iemand met coeliakie leidt het eten van gluten tot een afweerreactie in de dunne darm, met beschadiging van de darmwand als gevolg (*vlokatrofie*). Waarom het afweersysteem bij deze mensen op gluten reageert, is nog niet geheel duidelijk. Mogelijk ontwikkelen deze patiënten geen tolerantie voor gluten, of verliezen zij juist hun tolerantie.

HLA-DQ2 en HLA-DQ8

Er bestaan vele verschillende HLA-II-moleculen. Ze verschillen van elkaar qua vorm en dus ook qua functie. Elke vorm heeft een eigen naam gekregen. Gluten past precies op HLA varianten "DQ2" en "DQ8", die dan ook een belangrijke rol spelen bij coeliakie.

2 Ezelsbruggetje
Met wat fantasie is een HLA-II-molecuul voor te stellen als een ober met een presenteerblaadje, of "*HLA-DQ2/DQ8*", waarop alleen een glas cola van het merk "*gluten*" past. De ober presenteert dit glas cola aan zijn klanten. Er lopen echter ook politieagenten op het terras rond, de "*T-cellen*". Zij houden de boel in de gaten. Normaal levert het serveren van een colaatje van het merk "gluten" geen problemen op. Bij mensen met coeliakie zien de T-cellen de gluten als bedreigend (een verboden colamerk) en geven ze een seintje aan hun collega-afweercellen om de boel op te ruimen m.b.v. antistoffen tegen o.a. gluten (zie **Figuur 4**).

Figuur 4 *Gluten (1) bevinden zich, na een broodmaaltijd, in de dunne darm. HLA-DQ2/DQ8 (gele ober) presenteert gebonden gluten (2) aan de T-cel (politieman). De T-cel herkent: 'HLA-DQ+gluten' en geeft een signaal af aan collega B-cel (4). Dit is het begin van een afweerreactie, waarbij antistoffen tegen o.a. gluten worden aangemaakt (5&6).*



De diepte in

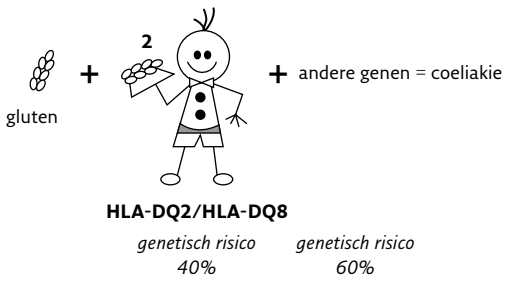
Nu gaan we de opgedane kennis over genetica en HLA-II-moleculen combineren. Inmiddels weten we dat we van elke genetische eigenschap twee allelen (varianten) hebben. Zo hebben we dus ook twee allelen die coderen voor twee varianten van HLA-II-moleculen, van beide ouders één. De verantwoordelijke genen liggen op chromosoom 6. Om te onderzoeken of iemand HLA-DQ2 en/of HLA-DQ8 heeft, worden alle genen die coderen voor de HLA-II-moleculen onderzocht.

Een combinatie van HLA-typen die kan voorkomen is bijvoorbeeld DQ2 met DQ4 (notatie DQ2/DQ4). DQ2 geeft risico op coeliakie, maar DQ4 niet. Deze persoon is *heterozygoot* voor HLA-DQ2 omdat hij/zij maar één kopie van DQ2 heeft, met daarnaast een andere variant van HLA-DQ. De combinatie DQ2/DQ2 betekent dat de persoon *homozygoot* is voor DQ2 (zie **Figuur 2**).

Risicoprofiel

Aanwezigheid van één HLA-DQ2 of -DQ8 molecuul (heterozygoot) geeft een klein verhoogd risico op coeliakie. Indien er sprake is van homozygositeit (dus twee maal HLA-DQ2 of HLA-DQ8) is het risico verder verhoogd. Daarnaast geeft HLA-DQ2 een groter risico op coeliakie dan HLA-DQ8, dus geeft de combinatie DQ2/DQ8 een hoger risico dan DQ8/DQ8, maar is het risico in het geval van DQ2/DQ2 het hoogst. Hoeveel hoger het risico precies is, verschilt per bevolkingsgroep. Dat het hebben van HLA-DQ2 en/of HLA-DQ8 geen 100% garantie geeft op het ontwikkelen van coeliakie komt doordat deze genen niet de enige genen zijn die een rol spelen in de ontwikkeling van coeliakie. Uit onderzoek is gebleken dat meer dan een kwart van de Nederlandse bevolking HLA-DQ2 en/of HLA-DQ8 heeft, zonder dat ze ooit coeliakie zullen ontwikkelen! De bijdrage van de HLA-II-genen aan het genetisch bepaalde risico op coeliakie is ongeveer 40%. De overige 60% wordt toegeschreven aan andere, voor een deel nog onbekende, omgevingsfactoren en genen (**Figuur 5**).

Figuur 5 *Coeliakie is een multifactoriële ziekte.*



Waarom vinden we het dan toch zinvol om een HLA-typering uit te voeren? Dat komt door de hoge *negatief voorspellende waarde* van de test. Hiermee wordt bedoeld dat een negatieve uitslag, dus afwezigheid van HLA-DQ2 en HLA-DQ8, betekent dat de kans op ontwikkeling van coeliakie verwaarloosbaar klein is. Het risico op coeliakie van een eerstegraads familielid van een patiënt met coeliakie zonder dat we weten wat het HLA-type van dit familielid is, wordt geschat op ongeveer 10%. Als dit familielid een HLA-typering laat doen, en hij/zij blijkt geen HLA-DQ2 en HLA-DQ8 te hebben, weten we dat dit familielid geen risico op coeliakie heeft (**Tabel 1**). Dit betekent dat verder onderzoek naar coeliakie bij deze persoon niet meer nodig is. Aan de andere kant is het verhoogde risico op coeliakie bij aanwezigheid van de genen voor HLA-DQ2 en/of –DQ8 een aanleiding deze personen (jaarlijks) te controleren op ontwikkeling van de ziekte. Zo hopen we coeliakie in een vroeg stadium te ontdekken, waarbij de gevolgen van het eten van gluten voorkomen kunnen worden door op tijd te starten met een glutenvrij dieet.

Tabel 1 *Risico op coeliakie geassocieerd met HLA-DQ2 en -DQ8, in de algemene populatie en bij eerstegraads familieleden van een patiënt met coeliakie.*

	DQ	Risico
Algemene populatie	DQ2 en/of DQ8 + DQ2 en DQ8 -	2% 0%
Eerstegraads familielid	DQ2 en/of DQ8 + DQ2 en DQ8 -	>15% 0%

Wat hebben wij nodig voor een HLA-typering?

Bijna al onze lichaamscellen bevatten DNA. Cellen die zich goed lenen voor DNA-onderzoek zijn de witte bloedcellen. Daarom wordt voor DNA-onderzoek meestal een buisje bloed afgenomen. Het is ook mogelijk om ander lichaamsmateriaal, zoals wangslijmvlies, te gebruiken. Helaas is de kans dat de HLA-typering mislukt dan een stuk groter.

Conclusie

Een HLA-typering is zinvol als u een eerstegraads familielid heeft met coeliakie. Als de uitslag positief is (HLA-DQ2 en/of HLA-DQ8 is aanwezig) zegt dit nog niet dat uw kind/u coeliakie zal ontwikkelen. Het risico is echter wel verhoogd. In dit geval raden wij aan om een normaal dieet te volgen en bij kinderen regelmatig screening op coeliakie specifieke antistoffen te laten verrichten. Indien de uitslag negatief is (HLA-DQ2 en HLA-DQ8 zijn afwezig), zal uw kind/u geen coeliakie ontwikkelen en is verdere controle niet nodig.

Handige websites;

- *www.coeliakiepoli.nl*, de website van de coeliakiepoli van het LUMC.
- *www.glutenvrij.nl*, de website van de patiëntenvereniging voor coeliakie.

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In de eerste plaats de kinderen met coeliakie en hun ouders, eerst in het LUMC in Leiden en later in het Rijnstate in Arnhem. Het moeten leven met deze chronische ziekte en de erbij horende dagelijkse beperkingen was en is nog steeds mijn belangrijkste motivatie om de zorg op basis van onderzoek te verbeteren.

Natuurlijk was het een voorrecht te mogen leren van zoveel mensen, die mij hebben gevormd op zowel medisch inhoudelijk, wetenschappelijk als menselijk vlak.

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CURRICULUM VITAE

MARGREET WESSELS werd op 5 april 1976 geboren in Almelo. In 1994 deed ze eindexamen VWO aan het OSG Erasmus te Almelo, waarna zij door middel van naplaatsing toegelaten werd tot de studie Geneeskunde aan de Rijks Universiteit Utrecht. Tijdens haar studie deed zij een onderzoeksstage in Vancouver, Canada, naar late complicaties van beenmergtransplantaties en deed zij een coschap dermatologie in het Tygerberg ziekenhuis te Kaapstad, Zuid-Afrika. Na haar coschappen werkte zij in 2001 eerst enkele maanden als consultatiebureau arts in Breda. Van oktober 2001 tot oktober 2003 werkte zij als ANIOS kindergeneeskunde in het Diakonessen Ziekenhuis te Utrecht en het Leids Universitair Medisch Centrum te Leiden, alwaar zij op 1 oktober 2003 startte met de opleiding tot kinderarts. Haar perifere stage deed zij in het Reinier de Graaf Gasthuis te Delft. In februari 2009 werd zij geregistreerd als kinderarts. Ze werkte vervolgens als waarnemend kinderarts in het Juliana Kinderziekenhuis te Den Haag tot aan de start van haar fellowship kindermaag-darm-leverziekten in het ErasmusMC te Rotterdam, locatie Sophia Kinderziekenhuis, in juni 2009. Vanaf 1 oktober 2010 zette zij dit fellowship voort in het Leids Universitair Medisch Centrum, gecombineerd met de start haar promotie onderzoek. Naast het onderzoek dat zij deed voor dit proefschrift, coördineerde zij de opzet van een biobank voor kinderen met coeliakie met gestandaardiseerde opslag van zowel biomateriaal als patiëntgegevens. Daarnaast was zij betrokken bij een onderzoek naar de uitkomsten van een eHealth systeem voor kinderen die onder poliklinische controle zijn voor coeliakie. Op 15 september 2012 werd zij geregistreerd als kinderarts-MDL. Sinds augustus 2013 werkt zij in die functie in het Rijnstate Ziekenhuis te Arnhem. Ze is er tot op heden wetenschappelijk actief voor haar promotie, maar ook daarbuiten. In december 2016 werd ze opleider Kindergeneeskunde in het cluster OOR-ON. Ze woont in Arnhem met haar man Menno Loos en hun kinderen Willemijn (2008), Sjoerd (2010) en Teun (2012).