Thesis for doctoral degree (Ph.D.) 2020

Coeliac disease in children and adolescents with type 1 diabetes – Screening, diagnosis and prevalence

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– SCREENING, DIAGNOSIS AND PREVALENCE

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Coeliac disease in children and adolescents with type 1 diabetes
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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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"Remain a beginner, like a child endowed with tremendous humility, patience and faith.
Such should be our attitude towards the experiences life brings to us.
Then we will keep on learning.
For the mind to grow and become as big as the universe, we should first become a child."

Amma Mata Amritanandamayi

To all my family, of blood and of heart ♥

A toda mi familia, la de sangre y la de corazón ♥

ABSTRACT

Background

Coeliac disease (CD) is more common in children and adolescents with type 1 diabetes (T1D). Both diseases share the same high-risk genes: human leukocyte antigen (HLA) DQ2 and DQ8. Other factors than gluten intake and high-risk genes are necessary to develop CD. In Sweden, there was a dramatic increase in CD in young, otherwise healthy, children between 1984 and 1996 and this has been called the "Swedish epidemic of coeliac disease", hereinafter referred as the Swedish CD epidemic. Over the last decade, the diagnostic guidelines for CD in children and adolescents have changed, but children with T1D are still not included in protocols to determine CD diagnosis without a biopsy, due to a lack of data.

Aims

The overall purpose of this dissertation was to expand current knowledge about CD in children and adolescents with T1D, with regard to the screening, diagnosis and prevalence of CD. One aim was to investigate the prevalence of CD in Swedish children and adolescents with T1D and compare the prevalence in individuals born before, during and after the Swedish CD epidemic. Another aim was to explore how CD screening in children and adolescents with T1D may be improved.

Research strategy

In Study I, we examined the medical records of 1,151 paediatric patients at a diabetes clinic in Stockholm to determine the prevalence of CD in children and adolescents with T1D, as well as the prevalence of CD in three subgroups. These were children born before, during and after the Swedish CD epidemic. In Study II, we investigated the prevalence of CD in patients with T1D at a Swedish national level, using several databases. We identified 1,642 children with T1D born during the Swedish CD epidemic (1992–1993) and 1,380 born after the epidemic (1997–1998). The total number of individuals born during these years was 430,374. In Studies III and IV, we used national cohort data from the Swedish prospective study Better Diabetes Diagnosis (BDD). In Study III, we analysed blood samples from 2,705 children and adolescents when they were diagnosed with T1D, to determine the links between HLA-DQ2 and HLA-DQ8, CD biomarker tissue transglutaminase (tTG) and diabetes autoantibodies. In Study IV, we analysed information from 2,035 children and adolescents with T1D, combined with data from the medical records kept by their diabetes clinics, to evaluate if high levels of tTG could predict CD. All the studies were approved by the Swedish Ethical Review Authority.

Results

Every tenth child and adolescent with T1D in Sweden also had CD. No difference in CD prevalence was found in children with T1D born before, during or after the Swedish CD epidemic. Many children were diagnosed with both diseases almost at the same time and the majority were diagnosed with CD within two years of being diagnosed with T1D. The CD biomarker tTG was related to the HLA high-risk genes DQ2 and DQ8, but not to diabetes autoantibodies. These risk-genes were absent in approximately 8% of the children with T1D. When the CD biomarker tTG was 10 times above the upper limit of normal, it was accurate in predicting CD in children and adolescents with T1D.

Conclusion

The prevalence of CD in children and adolescents with T1D in Sweden was shown to be one of the highest in the world. Children with T1D were not affected by different gluten intake recommendations in infancy, unlike the general population during the Swedish CD epidemic. This finding can be taken into account when planning both long-term observational studies and interventional studies about how to prevent CD. HLA was only useful in identifying the T1D population that was not at-risk of developing CD. We recommend repeated CD screening in children with T1D and HLA DQ2 and/or DQ8, and suggest that the first two years after their T1D diagnosis is the most important time. It is also suggested that guidelines for diagnosing CD in screened children should also apply to children with T1D, with regard to when biopsies can be avoided.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which can be found at the end of the thesis. The studies are referred to in the text using Romans numerals.

I. **Mara Cerqueiro Bybrant**, Eva Örtqvist, Sophie Lantz, Lena Grahnquist.
High prevalence of celiac disease in Swedish children and adolescents with type 1 diabetes and the relation to the Swedish epidemic of celiac disease: a cohort study
Scandinavian Journal of Gastroenterology, 2013 49:1, 52-58
DOI: 10.3109/00365521.2013.846403

II. **Mara Cerqueiro Bybrant**, Elsa Palmkvist, Marie Lindgren, Hanna Fischerella Söderström, Fredrik Norström, Hans Hildebrand, Annelie Carlsson.
Prevalence of coeliac disease in children with type 1 diabetes during and after the Swedish epidemic of coeliac disease
Manuscript

III. **Mara Cerqueiro Bybrant**, Lena Grahnquist, Eva Örtqvist, Cecilia Andersson, Gun Forsander, Helena Elding Larsson, Åke Lernmark, Johnny Ludvigsson, Claude Marcus, Annelie Carlsson, Sten A Ivarsson.
Tissue transglutaminase autoantibodies in children with newly diagnosed type 1 diabetes are related to human leukocyte antigen but not to islet autoantibodies: A Swedish nationwide prospective population-based cohort study
Autoimmunity, 2018, 51:5, 221-227
DOI: 10.1080/08916934.2018.1494160

IV. **Mara Cerqueiro Bybrant**, Elin Udén, Filippa Frederiksen, Anna L. Gustafsson, Carl-Göran Arvidsson, Anna-Lena Fureman, Gun Forsander, Helena Elding Larsson, Sten A. Ivarsson, Marie Lindgren, Johnny Ludvigsson, Claude Marcus, Auste Pundziute Lyckå, Martina Persson, Ulf Samuelsson, Stefan Särnblad, Karin Åkesson, Eva Örtqvist, Annelie Carlsson.
Celiac disease can be predicted by high levels of tissue transglutaminase antibodies in children and adolescents with type 1 diabetes
Accepted for publication in Pediatric Diabetes
Manuscript ID PDI-20-O-0309.R1

CONTENTS

LIST OF ABBREVIATIONS

AGA	Anti-gliadin antibody
BDD study	Better Diabetes Diagnosis study
CD	Coeliac disease
CI	Confidence interval
DPG	Deaminated gliadin peptides antibodies
EliA	Enzyme linked immuno-assay
ELISA	Enzyme-linked immunosorbent assay
EMA	Endomysium antibody
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
GADA	Glutamic acid decarboxylase antibody
HLA	Human leukocyte antigen
IA-2A	Islet antigen 2 antibody
IAA	Insulin autoantibody
IgA	Immunoglobulin A
ISPAD	International Society for Pediatric and Adolescent Diabetes
NDR	National Diabetes Register
NICE	National Institute of Health and Care Excellence
Swediabkids	A part of the Swedish National Diabetes Register
T1D	Type 1 diabetes
tTG	Anti-tissue transglutaminase antibody
ULN	Upper limit of normal
WHO	World Health Organization
ZnT8A	Zinc-transporter 8 autoantibody

1 PREFACE

Type 1 diabetes (T1D) and coeliac disease (CD) are two very common chronic autoimmune diseases in children and adolescents. Over the past three decades, research about these diseases, and the combination of them, has intensified worldwide. At the beginning of 2005, when the first study included in this thesis was designed, there was no Swedish or international consensus about screening for CD in children and adolescents with T1D.

The screening methods that were available internationally were certainly getting better, with more accurate tests and better techniques for taking biopsies. Despite this, concerns had been highlighted about providing additional diagnoses of CD to children who already had T1D and this situation had led to comprehensive discussions by paediatric endocrinologists and gastroenterologists. It was against this background that I approached the research field of CD in T1D and came to play an active role in the design and execution of the studies included in this thesis.

This thesis summarises four separate studies on children and adolescents with T1D and CD. The first part of the thesis provides a general introduction to the field. The next part compiles and discusses the results of these studies with regard to screening methods, diagnostic procedures and the prevalence of CD. The four original research studies, on which this thesis is based, are also included.

Mara Cerqueiro Bybrant

2 BACKGROUND

2.1 COELIAC DISEASE

2.1.1 History

The history of CD contains interesting milestones and the first description of the disease dates back to around the second century AD. It emanated from the Fertile Crescent, which is an area between the Tigris, Euphrates and Upper Nile rivers. This was where the first cultivation of cereals, particularly wheat and barley, was recorded during the Neolithic period. The development of agriculture and cooking radically modified human diets, as up to that point they had relied on hunting and gathering. As far as we know, the first well-described symptoms of CD were provided by Aretaeus of Cappadocian (circa 120-180 AD), who was a notable Greek physician. He described the causes and signs of several diseases, including the disease we now call CD. The origin of the word coeliac derives from the Greek word *koiliakós*, which was used to refer to the intestinal involvement in this abdominal disease. When Aretaeus' texts were found and translated, the word coeliac was broadly adopted (1, 2).

Table 1, on the next page, summarises the most relevant scientific advances in CD throughout history.

2.1.2 Definition

CD is an immune-mediated disorder, which is triggered by exposure to gluten and related prolamins in genetically susceptible individuals (3, 4). It is characterised by clinical manifestations that are not always overtly present, CD biomarkers, such as specific antibodies against tissue transglutaminase, genetic markers such as human leucocyte antigen (HLA)-DQ2 and HLA-DQ8, and small intestine enteropathy (3).

The Oslo definitions for CD-related terms, published in 2013, were a multidisciplinary attempt to evaluate the suggested definitions and terminology used to define the different presentations and forms of CD (5).

The definition of symptomatic CD was clinical evidence of gastrointestinal and/or extraintestinal symptoms that were attributable to gluten. The so-called classical CD presentation included the manifestation of clinical signs and symptom of malabsorption. As malabsorption in CD is a result of the destruction of the mucosa in the small intestine, which stops the intestine from absorbing nutrients as usual, some of the symptoms in the paediatric population are loose stools (diarrhoea and steatorrhoea), abdominal distension, weight loss, failure to thrive and iron-deficiency or anaemia (5).

YEAR	MILESTONES OF COELIAC DISEASE
A.D. 120-180	First description of the disease by Aretaeus of Cappadocian. The Greek word *koiliakós* , meaning those who suffer because of the intestine, was used to describe a clinical syndrome characterized by chronic diarrhoea, paleness and malnutrition.
1888	Paediatrician Samuel Gee described an intestinal condition, related to eating flour, which he defined as "Coeliac affection". Gee suggested that CD should be treated by regulating a person's diet.
1924	Dr Sidney Hass proposed a diet for CD, based on reducing carbohydrate intake.
1950	Paediatrician Willem-Karel Dicke observed that children with CD improved when wheat, rye and oatmeal were excluded from their diet.
1952	Dr Charlotte Anderson and her team discovered that gluten dough was the toxic component that caused CD. Since then, the gluten-free diet has formed the basis for treating CD.
1954	Dr John W Paulley provided the first accurate description of the coeliac lesions in the mucosa of the bowel with the characteristic villous atrophy of CD.
1957	Dr William H Crosby and Heinz W Kugler described how to carry out intestinal biopsies using endoscopy.
1964	The discovery of the existence of circulating antibodies by Berger suggested an immune component.
1969	The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) established strict diagnostic criteria for CD for the first time. This was called the Interlaken criteria or rule of three biopsies. It required one diagnostic biopsy, one with a mucosa normalization and another after a provocation test.
1986	Dr Michael N Marsh described the histopathological stages of CD mucosal damage.
1990	New ESPGHAN criteria for the diagnosis of CD allowed clinicians to establish a CD diagnosis with a single biopsy in children who met the specific criteria.
1999	Pathologist Dr Georg Oberhüber presented a variation on the Marsh stages of CD.
2012	The ESPGHAN diagnostic criteria were reviewed and specific criteria were established, allowing a no biopsy approach to CD diagnosis.
2013	The Olso definitions of CD were published and these divided symptomatic and asymptomatic CD. They identified potential CD, and individuals at-risk, and established gluten intolerance with negative serology as a separate entity.
2019	The new ESPGHAN guidelines for diagnosing CD were presented, amplifying the criteria on the no biopsy approach, providing the possibility to diagnose CD in screened children without symptoms.

Table 1. *Milestones in the history of CD.*
Modified with permission from Coronel Rodriguez et al (6).

This differed from the definition of non-classical CD that included patients presenting without symptoms of malabsorption. The term subclinical CD is now deemed more acceptable than silent CD for individuals without signs or symptoms of CD (5).

Furthermore, CD autoimmunity in an individual was defined as an increased level of CD biomarker at least twice, while potential CD corresponded to CD autoimmunity with a normal small intestinal mucosa. In addition, people who were genetically at-risk for CD were described as individuals with positive genetic tests for either of the CD risk genes (5).

The definitions and diagnosis of CD in the population have usually been represented with the iceberg model (Figure 1).

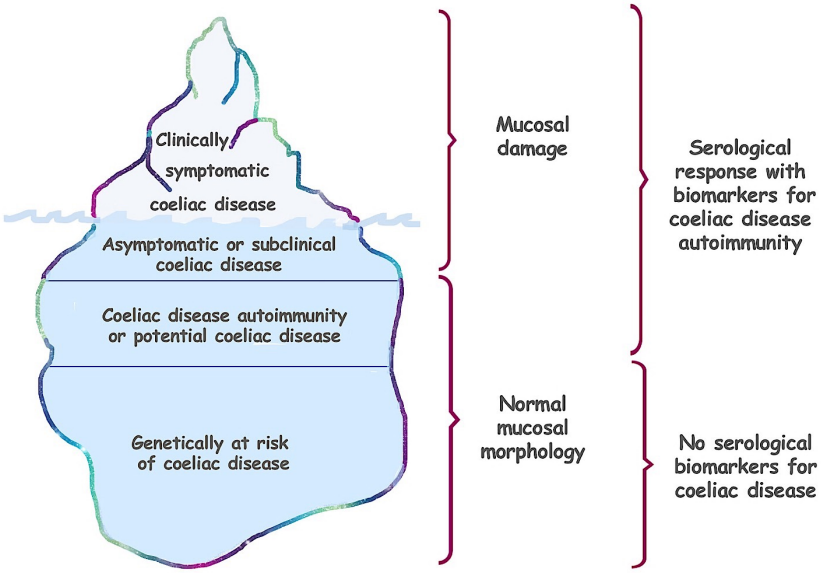


***Figure 1**. The iceberg model. The known CD cases are in the visible part of the iceberg, while the submerged part of the iceberg represents undiagnosed cases.*

2.1.3 Incidence and prevalence

CD is a common disease that affects individuals at all ages worldwide. About one in 100 people in the western world have the disease (7) The number of people that are diagnosed with CD depends on several different factors. The prevalence of CD also varies within the same populations, which, in some cases, may depend on the design of the study to find individuals with CD, as many people with CD may have an undiagnosed disease (8).

The prevalence of CD varies with regard to sex, age and location. CD is more common in females, as two out of three patients are girls or women (9-11). However, this sex disparity may be lower when the population is screened (8). CD can start at any age, although two peaks of CD onset are usually described: one in early childhood around the age of two and the other around the age of 30. In Sweden, the median age for a CD diagnosis in children and adolescents has increased from 1.0 year of age in the 1970s to 6.8 years in 2009 (11). In addition, the number of children with CD varies in different regions, within the same country (12, 13) and between neighbouring countries (14). Moreover, CD is increasing over time in different parts of the world (15).

Sweden has one of the highest prevalence figures of CD in the world (7). The disease is also one of the most common chronic diseases in children and young people in Sweden. A study that screened Swedish school children for CD found a prevalence of 3% (16). Furthermore, a prospective study carried out in four different countries that followed children who were genetically at-risk, showed that Swedish children had nearly double the risk of developing CD than North American children (17).

2.1.4 Swedish epidemic of coeliac disease

In Sweden, there was a dramatic increase in CD in young children between 1984 and 1996, when the number of cases of CD quadrupled. This period of time has been called "The Swedish epidemic of coeliac disease", hereinafter referred as the Swedish CD epidemic (18-21).

Paediatricians throughout Sweden diagnosed an increasing number of young children with CD. The cumulative incidence of CD reached higher levels than those previously reported, from one to four cases per 1,000 births. This increase in incidence, especially among children under two years of age, was followed by an abrupt decline (12, 18) (Figure 2).

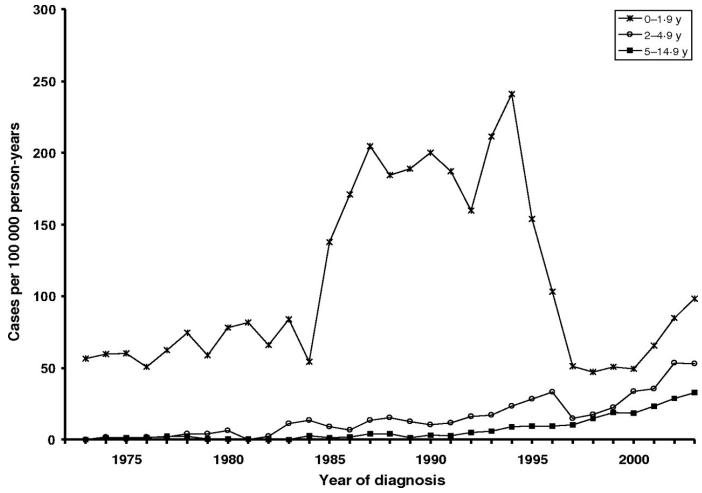


***Figure 2**. Incidence of CD per 100,000 person-years, illustrating the incidence of CD in different ages from 1973-2003. Printed with kindly permission from Olsson et al and the publisher (12).*

During the Swedish CD epidemic the Swedish infant feeding recommendations changed with regard to time for gluten introduction. The national recommendations postponed gluten introduction from four to six months of age, which was the same period when breastfeeding was more likely to be discontinued. At the same time, the gluten content in infant milk cereals drinks and porridges was increased, in a move that was unrelated to the changes in the national recommendation (10).

In 1996, the feeding recommendations changed again and they were almost the same as they were before 1986. These changes included a gradual introduction of gluten, preferably during breastfeeding, as well as a reduction in the gluten content of commercially available products for infants (18). After these new recommendations were implemented, the incidence of CD rapidly decreased, to similar levels as before the epidemic (10). These fluctuations stimulated research about triggers for the development of CD, as well as strategies for primary prevention (12).

2.1.5 Diagnosis

Serology

The diagnosis of classical CD has been based on the clinical presentation, with signs and symptoms, the presence of CD biomarkers in peripheral blood and small intestine mucosal damage (22).

During the few past decades, the tests for CD biomarkers have been improved. The CD biomarkers, which are based on serology, include the autoantibodies that have been showed to be present in high levels when the mucosal damage exists (23). One of the first tests that was made available focused on anti-gliadin antibodies (AGA). These were not as specific as the later tests that targeted endomysial autoantibodies (EMA). The accuracy of CD biomarkers has increased since tissue transglutaminase (tTG) immunoassays were introduced in the late 1990s (5, 24). These tests are constructed as immunoglobulin A (IgA) tests. In children with IgA deficiency, deaminated gliadin peptides antibodies of the immunoglobulin G (DGP) type may be used to diagnosis CD. The 2020 guidelines from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) provided forest plots about the sensitivity and specificity of the available tests (22).

In summary, the antibodies that have been the commonly used as CD biomarkers are as follows:

- AGA antibodies: these are not very specific, but they were useful before the development of other CD biomarkers.
- EMA antibodies: these target the enzyme transglutaminase which make them very specific, but less sensitive. They can be used as a confirmatory test due to their high specificity.
- tTG antibodies: these show high sensitivity and specificity and their levels can be correlated with the degree of intestinal mucosal damage found in biopsies. They are the first choice due to their high sensitivity and good specificity.
- DGP antibodies: these are directed against gluten fragments that have been deaminated by the tissue transglutaminase enzyme in the intestine. The antibody types that are most frequently used are those of the immunoglobulin G class, especially for IgA deficiency patients.

Biopsy

The purpose of biopsies is to identify the small intestine mucosal damage that is characteristic of CD. The pathological changes in the mucosa include increased number of intraepithelial lymphocytes, crypt hyperplasia and villous atrophy, which classification were described by Marsh (23, 25). These changes can only be observed by intestinal biopsies that are obtained after an upper gastrointestinal endoscopy. An older methodology was capsule biopsy, which is seldom used today. The assessment of histological damage is mostly graded according to the Marsh-Oberhüber criteria. These criteria report the degree of injury including inflammation and flattening of the villi of the mucosa in the small intestine (26, 27) (Figure 3, on the next page).

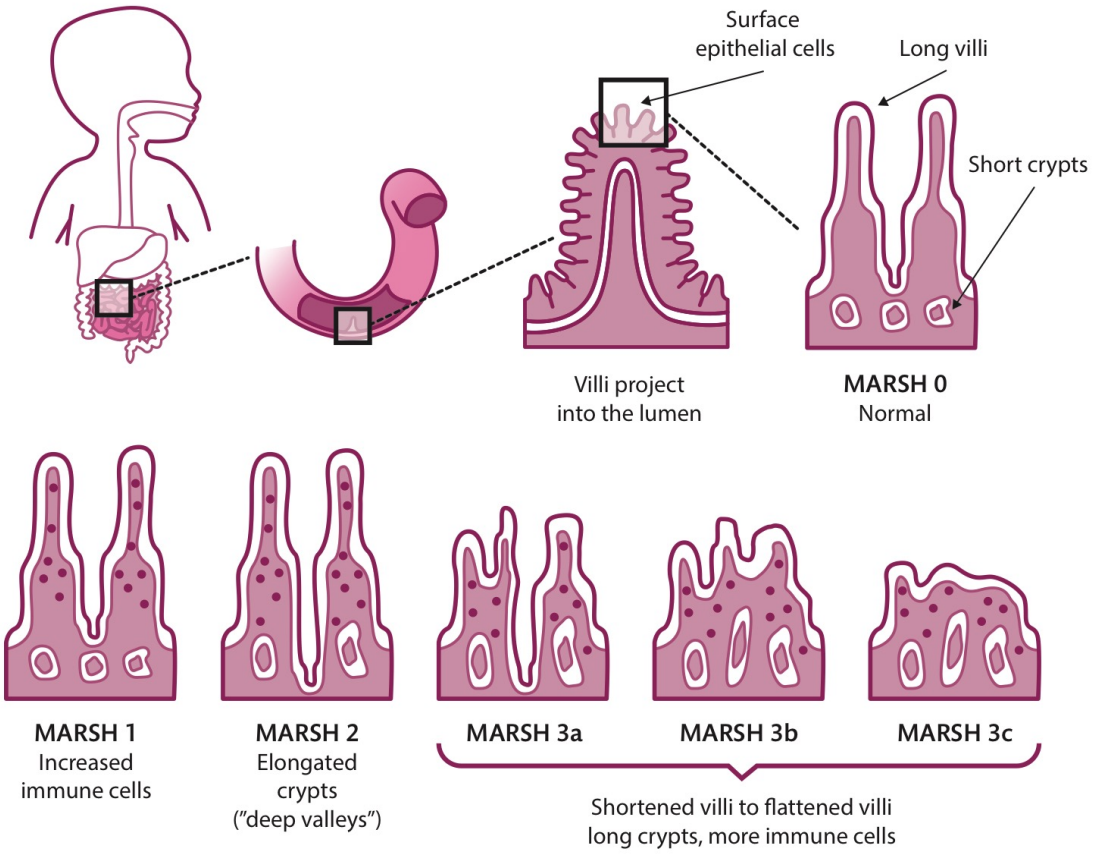


Figure 3. *Schematic representation of mucosal damage to the small intestine.*
Marsh 0 represents normal mucosa with long villi and short crypts in the epithelial cells.
Marsh 1 shows increased immune cells (intraepithelial lymphocytes).
In Marsh 2 there are aggregating elongated crypts, so-called "deep valleys".
Marsh 3a/b/c correspond to different stadia of shortened villa, to complete villus atrophy, accompanied by more autoimmune cells and long crypts.
Illustration inspired by "Celiakiboken" (28).

However, it is worth mention that small bowel involvement could be patchy (areas with different degrees of damage) which makes a normal histological grade not a completely sure way to overruled CD in all cases (29, 30). These so called false negative biopsies have been discussed in the literature in the past decade (31, 32), and have encouraged other diagnostic approaches. One of the most important of these is the need to take several biopsies from both proximal and distal parts (22). Furthermore, histological damage is not always related to the patient's symptoms, that is, more damage does not translate into more symptoms, even if a correlation has been seen regarding CD biomarkers (23).

Guidelines

The first European diagnostic criteria for CD were introduced by ESPGHAN in 1969 (29, 33). The next revision occurred in 1990, allowing clinicians to abandon the ESPGHAN 1969 criteria of three mandatory biopsies (30).

The later the publication of the ESPGHAN 2012 guidelines marked a change in the diagnosis of CD. It opened up a new possibility in one of the algorithms: the option for clinicians to provide a CD diagnosis without an intestinal biopsy was accepted under certain circumstances (3). The no biopsy approach was recognised after reviewing the literature and noting that the serological tests showed an increase in sensitivity and positive predictive values (PPV) (34, 35).

The criteria for allowing a no biopsy diagnosis were the following: tTG level 10 times above the upper limit of normal (ULN), a second test with elevated EMA and the presence of HLA risk genes (3).

These criteria were only applicable for the children with symptoms. They did not consider children and adolescents with T1D, regardless of tTG levels, who were usually identified through screening, or among other groups of asymptomatic children (3).

After the 2012 ESPGHAN guidelines were published (3) other international authorities chose to follow the recommendations, as stated in the United Kingdom guidelines (36), or argue against the no biopsy approach, as the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition indicated in a clinical report (4).

In the following years, several studies applied the ESPGHAN guidelines to ongoing, or retrospective, studies. New data on asymptomatic children, and how they could be included into the guidelines, were published (37-42). Most of these studies, and the others that were included in the assessment that led to the ESPGHAN 2020 guidelines (22), showed that CD could be diagnosed by the no biopsy approach, even in asymptomatic children. There were no studies with a high number of individuals with T1D in the ESPGHAN 2020 analysis and that was why these new guidelines were not able to address the possibility of a no biopsy approach for children and adolescents with T1D (22).

It is important to understand that small bowel biopsies are by no means outdated as they still play a central part in diagnosing CD. For example, analysing biopsies is mandatory in children and adolescents with IgA deficiency or if the level of CD biomarkers does not meet the established criteria (22).

2.1.6 Treatment

CD cannot be cured and the only treatment that is currently available is a lifelong gluten-free diet. Gluten is a protein found in various grains, such as wheat, rye and barley (5). The term gluten-free is defined by authorities both in Europe and North America and this covers foods items without the wheat proteins (gliadins and glutenins), barley (hordeins) and rye (secalins) and other hybrids, such as triticale cereals (43, 44).

Most of the individuals with CD who follow the dietary advice they are given recover, because the intestinal mucosal damage heals when they avoid gluten. In the Oslo definitions,

persistent or recurrent symptoms due to malabsorption, despite a gluten-free diet for more than 12 months, was defined as refractory CD (5). This entity is very uncommon, as it only affects 0.3% of all patients diagnosed with CD, and it is even more uncommon in children and adolescents (45).

Undetected or untreated CD has been associated with a number of symptoms and complications. This complications include iron-deficiency, retarded height and weight development and delayed puberty in children and adolescents. Some of the long-term complications are osteoporosis, depression, and, in rare cases, an unusual type of bowel cancer (lymphoma), and lower fertility or infertility (8, 46).

2.1.7 Immunological pathogenesis

CD is an autoimmune disease that primordially affects the small intestine mucosa (45). In CD, the initiators of the autoimmune cascade are the gluten peptides that are partially digested into gliadins. In the lumen of the intestine, these peptides are carried across the epithelial barrier and deaminated by tissue transglutaminase enzymes. Deamidated gliadin peptides are subsequently recognized by, and bind to, cells porting HLA-DQ2 and/or HLA-DQ8 antigens in their surface, the so-called antigen presenting cells (47).

Under distinctive proinflammatory conditions, these antigen presenting cells are recognised by T cells that trigger the immune system activation. This activation promotes a maturation of B cells producing IgM, IgG and IgA antibodies against gliadin and tissue transglutaminase. In addition, T cells are also involved in the production of pro-inflammatory cytokines (interleukin-15, interferon γ and tumour necrosis factor α), which, in turn, probably further increase gut permeability and may accelerate the initiation of the enteropathy (48).

2.1.8 Multifactorial aetiology

The multifactorial aetiology of CD is a complex combination of, and interaction between, genetic and environmental factors (48). The adverse reaction to gluten can only occur if the high-risk genes are present (49, 50). However, having the high-risk genes HLA-DQ2 and/or HLA-DQ8, and being exposure to gluten, is not enough to develop CD. Other factors that determine whether someone develops CD appear to be involved in triggering the onset of the disease, but these are unknown to great extent (51) (Figure 4, on next page).

In common with many others autoimmune diseases, CD has a strong hereditary component. It has been shown to occur in 10% of family members, and the concordance rates among monozygotic twins is approximately 50-80% (52, 53).

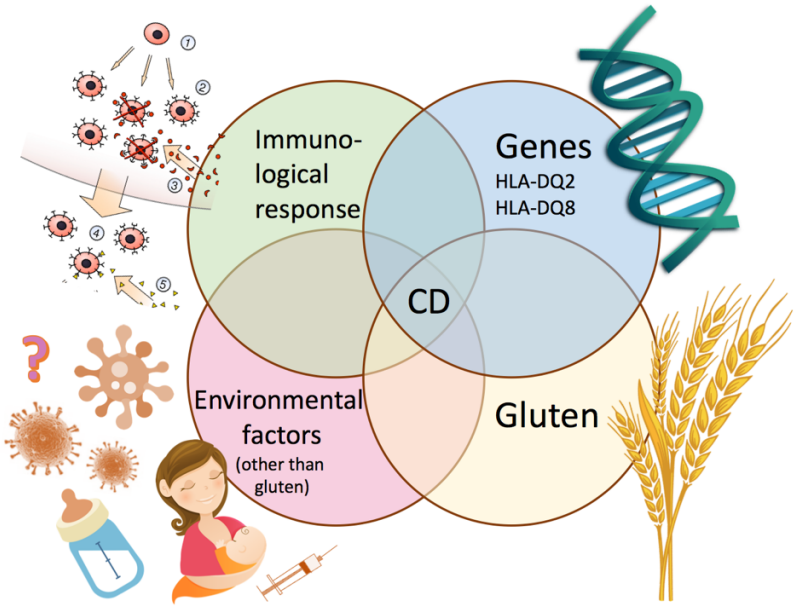


***Figure 4**. In the aetiopathogenesis of CD, genetic and immunological factors interact in response to environmental factors, where a necessary but not sufficient factor is the intake of gluten. Figure inspired by "Focus in CD" (54).*

The greatest genetic contributors are the previously mentioned HLA high-risk genes, located on the short arm of chromosome 6 (locus 6p21) (53). The genotype HLA-DQ2/DQ2 confers the most risk for developing CD (50). The HLA genotypes encode major histocompatibility complex (MHC) cell-surface membrane glycoproteins that bind antigens to present them to T cells receptors. In a figurative way, the risk-genes code for a type of presentation tools, as a "lock", that allows the modified gluten-fragment (deaminated gliadin), the "key" to be tightly bound, and thus these complexes can be misinterpreted as dangerous and therefore activate the immune system (54, 55) (Figure 5).

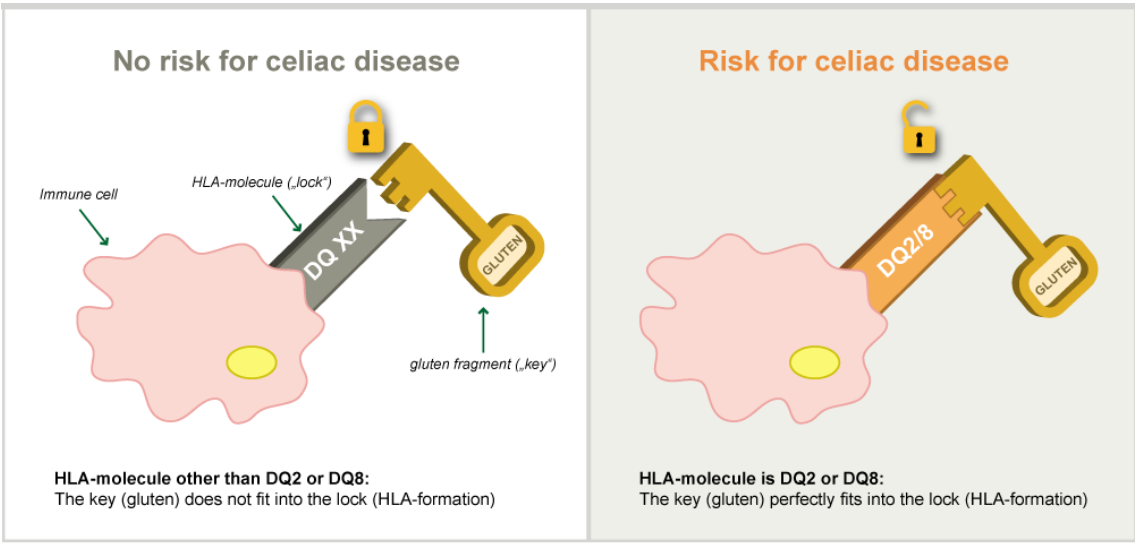


***Figure 5**. HLA DQ2/DQ8 and its role in the development of CD (simplified illustration). If HLA DQ2/DQ8 is not present (left), the gluten-fragment is not tightly bound by the HLA molecule on the antigen presenting cell and does not initiate any immune reaction. If HLA DQ2 and/or DQ8 are present (right), the gluten-fragment is bound firmly and an immune reaction may be provoked if other yet unknown conditions do also apply.*
Printed with kindly permission from Prof. Sybille Koletzko, "Celiac facts" (54).

Moreover, there are many other non-HLA-related genes that have been shown to be related to a higher risk for CD through genome-wide associations. Many of these genes have connections to T cells and B cells, which also contribute to the development of CD, even if the relevance of each of them seems to be limited or has a modest effect (56).

With regard to environmental factors, the specific significance of these is still not well understood (14, 57). Some dietary factors that were recognized during observational studies were then well-studied in prospective and interventional multicentre studies. Early studies suggested that breastfeeding could reduce the risk of CD. However, two interventional studies in 2014 were unable to demonstrate that breastfeeding, or the time of gluten introduction in infancy, were effective in preventing CD (58, 59). These studies were randomized trials on children who had a high-risk of developing CD. In contrast, in 2019, two newly published cohort studies (60, 61) have suggested that the amount of gluten may actually be associated with future CD autoimmunity and CD development. The 2014 and 2019 studies both included at-risk child populations. Nevertheless, there has only been one truly population-based study that focused on the duration of breastfeeding and when and how much gluten was introduced and that was a study from Norway, also published 2019 (62). The main finding of that study was that the risk of CD increased with each gram of extra gluten intake at the age of 18 months. Furthermore, the authors reported a higher risk when gluten was introduced after six months of age and stated that children with a longer period of breastfeeding had a lower intake of gluten in infancy. Interestingly, they noted a stronger association between the amount of gluten and the risk for CD in children with intermediate or low risk HLA for CD (62).

Other factors related to pregnancy and the perinatal period have been explored in longitudinal studies. The assessments included parental smoking, maternal gluten intake and maternal drug consumption, but iron intake was the only factor that showed a correlation to a higher risk for CD. Even birth weight and the season of birth have been explored, but these factors did not contribute to major risks (14).

Associations, and lack of associations, have been reported for various infectious diseases and their roles in triggering CD. In particular, gastrointestinal infections and the repeated use of antibiotics could play a potential role in the development of CD (14). The possible mechanism could be changes in the microbiome and the gastrointestinal micromilieu, which may be involved in the pathogenesis of CD (14). In a recent study, antibiotics treatment during the first year of life was positively associated with CD diagnosis in the Danish and Norwegian cohorts (63).

In a similar way, vaccines may indirectly influence the risk of infections and the later risk of CD, but a Swedish study did not find a correlation between early vaccinations and the risk of CD (64). Particular attention has been given to the oral rotavirus vaccine. One population-based cohort study found no higher risk of developing CD after vaccination (65). In addition, a randomized control trial reported that the prevalence of CD was lower in the rotavirus vaccinated group and it was suggested that the wild form of the virus may trigger CD (66).

2.2 TYPE 1 DIABETES

2.2.1 History

Diabetes is a disease that has been known since ancient times. The first reference appeared in the Ebers Papyrus (1500 BC). This Egyptian medical papyrus contained information about treatment for the condition's main symptom: polyuria (67).

In Hindu medicine, sticky urine with a sweet smell was described in the Vedas and called "madhumeha" (urine of honey). Sushruta, the father of Hindu medicine, distinguished a disease form that occurred in young people, leading to death, and another type that occurred in the elderly (1).

In the second century A D, Aretaeus of Cappadocian described diabetes patients through their urinary symptoms, stating that "the sick never stop urinating". He called this disease "diabetes", from the Greek word *siphon*, "to run through" (67).

Table 2, on the next page, briefly describes T1D in terms of the historical milestones and scientific advances.

2.2.2 Definition

Diabetes is a group of multifaceted metabolic disorders that cause chronic, elevated blood glucose levels. The definition of T1D is based on how diabetes is diagnosed.

The latest guidelines from the International Society for Pediatric and Adolescent Diabetes (ISPAD) (68) state that: "*The term diabetes mellitus describes a complex metabolic disorder characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both".* This definition is in accordance with the 2007 definition from the American Diabetes Association (69), which also specifies that T1D is caused by an absolute deficiency of insulin secretion and that individuals at-risk for T1D can "*be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers".*

2.2.3 Incidence

T1D is the most common serious chronic disease in Swedish children (70). Sweden has the second highest incidence of T1D in the world, after Finland (71, 72). Around 50,000 individuals have T1D in Sweden and about 7,000 are children and young people. About 800 children are diagnosed with T1D in Sweden every year (73).

In contrast to other autoimmune diseases, T1D affects slightly more boys in most populations (9). This male predominance is clear in Swedish children (74).

YEAR	MILESTONES OF TYPE 1 DIABETES
1550 B.C.	The first description of diabetes was found in the Egyptian medical Ebers Papyrus.
6th Century B.C.	Ancient Hindu ayurvedic physicians called the disease *madhumeha* ("honey urine"). Sushruta identified two types: in the young and in the elderly.
200 A.D.	Aretaeus the Cappadocian coined the word *diabetes* in Greek, "siphon", which means "to run through".
1670	Thomas Willis adopted the name of diabetes mellitus.
1775	Mathew Dopson described how excess blood sugar was the cause of excessive sugar in the urine.
1869	Paul Lagerhans described the islet cells in the pancreas.
1889	John Von Mering and Oscar Minkowski discovered that removing the pancreas caused diabetes symptoms in animals.
1921	Frederick Grant Banting and Charles H Best, working in John Macleod's laboratory at the University of Toronto, succeeded in isolating insulin and treating a diabetic dog. Insulin was subsequently purified by James B Collip.
1922	Leonard Thompson, a 14-year-old boy with diabetes, was given the first injection of insulin at the Toronto General
1923	The Nobel Prize in Physiology or Medicine 1923 was awarded jointly to Frederick Grant Banting and John James Rickard Macleod "for the discovery of insulin".
1966	The first-ever pancreas transplant was performed at the University of Minnesota by surgeons Richard Lillehei and William Kelly.
1970s	Continuous subcutaneous insulin infusion was introduced and the first insulin pumps were tested.
1980s	Introduction of insulin pens.
1999	The first device for continuously reading blood glucose levels was approved.
2010s - 2020s	Advanced diabetes technology improved. Applications supporting data to improve monitoring and treatment were created. Closed-loop insulin delivery, the so-called artificial pancreas, was developed.

Table 2. *Milestones about the history of T1D (1, 67, 75).*

A rise in the incidence of T1D has been seen globally in the last few decades. About 96,000 children are diagnosed with T1D in the world each year (72). Trends in the incidence of T1D vary markedly from country to country (68), possibly due to genetic variations and environmental differences in different populations (72). In Sweden, almost double number of children now develops T1D compared to 1980 (71, 73) (Figure 6, on the next page).

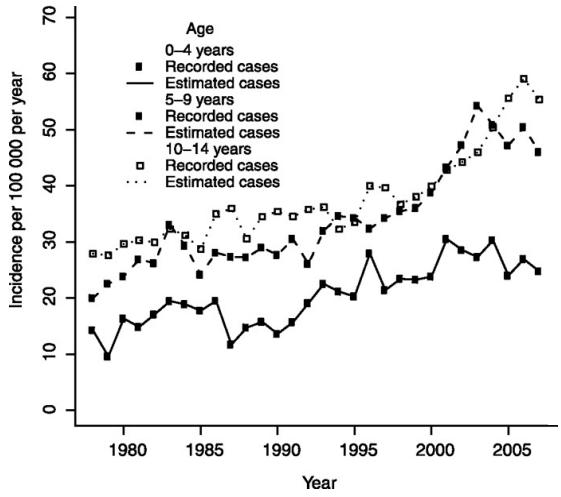


***Figure 6**. The increased incidence of T1D in Sweden according to age at diagnosis.*
Reprinted with kindly permission from Berhan et al and the publisher (73).

Two major hypotheses for the increasing incidence of T1D have been suggested. One is the hygiene hypothesis, which suggest that urban environments lack the microorganisms that used to stimulate the immune system. This loss of stimuli would result in an inappropriate immune activity seen in autoimmune diseases such as T1D (76). The other is the accelerator hypothesis, which advocates that insulin resistance and hyperglycaemia metabolically upregulate ß-cells, leading to glucotoxicity that accelerates the ß-cell loss and causes T1D in genetically susceptible individuals (77). Further investigations are still needed to validate these theories. Multiple trials aiming to prevent T1D development are ongoing or being planned which may help to identify additional causes (78).

2.2.4 Diagnosis

The classic symptoms that appear during the onset of T1D are increased thirst, large amounts of urine and weight loss. Other signs are fatigue and blurred vision (68).

T1D is diagnosed by blood tests and blood glucose levels and tests for diabetes autoantibodies can help to distinguish between different types of diabetes (68, 69).

An exact definition of blood glucose levels and diabetes has been provided by ISPAD (68): *"A marked elevation of the blood glucose levels confirms the diagnosis of diabetes, including a random plasma glucose concentration ≥11.1 mmol/L (200 mg/dL) or fasting plasma glucose ≥7.0 mmol/L (≥126 mg/dL) in the presence of overt symptoms."*

2.2.5 Treatment

T1D cannot be cured at the moment. The only treatment that is currently available is to add insulin. The treatment goal is that the blood glucose level stabilises and stays within normal limits (70).

Technological support has been at the forefront of research over the past decade (75). These technical advantages include insulin pumps and continuous glucose monitoring and the

ability to communicate glucose values to various applications. In addition, loop systems have been developed, which means that the blood glucose level in a sensor can help the insulin pump to decide the amount of insulin that is delivered (75).

2.2.6 Immunological pathogenesis

T1D is an organ-specific autoimmune disease that affects the pancreas. The pathogenesis of T1D is believed to include T cell activation, possibly through the presentation of modified peptides in the pancreas. Activated T cells promote the destruction of the insulin-producing ß-cells in the pancreas, which usually results in absolute insulin deficiency (48, 68).

There are several antibodies that are important in T1D, but their roles seems to be related to the consequences of T1D, rather than the root causes. Autoantibodies to antigens of the pancreatic ß-cell are the first sign of disease and have been used as a predictive marker of the immunological process (79).

The autoimmune markers involved in T1D include the 65KDa isoform of glutamic acid decarboxylase autoantibodies (GADA), insulinoma-associated-2 autoantibodies (IA-2A), insulin autoantibodies (IAA) and three types of zinc transporter-8 autoantibodies (ZnT8A). The presence of one or more of these autoantibodies, in addition to the clinical presentation, confirms T1D (68). Earlier studies have shown that approximately 93% of Swedish children with newly diagnosed T1D have at least one of these autoantibodies (80, 81).

2.2.7 Multifactorial aetiology

The multifactorial aetiology of T1D is a complex combination of, and interaction between, genetic and environmental factors that are unknown to great extent (68) (Figure 7).

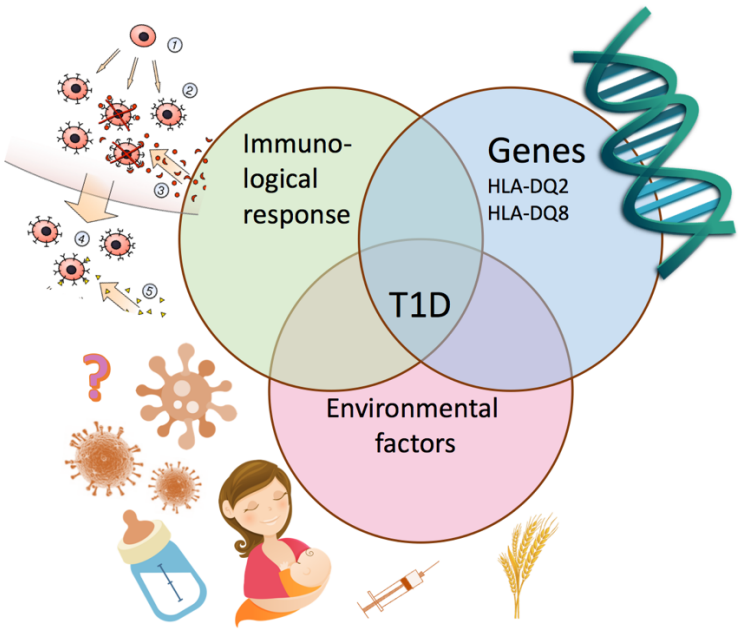


Figure 7*. In the aetiopathogenesis of T1D, genetic and immunological factors interact in response to environmental factors. Figure inspired by "Celiac Facts"(54) and adapted to T1D.*

Several genes have been linked with T1D by genome-wide association, and these include both highly susceptible and highly protective haplotypes (82). The HLA-DQ alleles have a well-established association to T1D risk (83, 84). The HLA region is responsible for up to 50% of the genetic risk. The HLA-DQ2 and HLA-DQ8 haplotypes, alone, or in combination, are the strongest known genetic determinants for T1D and they confer a 5% absolute risk of diabetes at the age of 15 (85). Nearly 90% of the Scandinavian paediatric T1D population have one, or both, of these haplotypes (86). However, less than 10% of those children with the highest risk genotype (HLA-DQ2 or DQ8) will develop clinical diabetes (87).

The complex genetic background of T1D also involves loci outside the HLA region (82). More than 40 non-HLA genes have been identified, including the INS insulin gene, PTPN22 gene and CTLA4 gene (87), which all code interactions with the immune system. However, their effects on the pathogenesis is much smaller, and it has been suggested that they only modify the risk established by the HLA genotype (46).

When it comes to the effect of the environment, two factors have particularly been associated with T1D in epidemiological and immunological studies. One is the exposure to enteroviral infections and the other is cows' milk, latter with conflicting results (88). Other theories have included Vitamins D and E, while iron intake during pregnancy and in early childhood have not shown an association (89). These nutritional and infectious causes, and others, have been described in the literature, but their specific significance are still mainly undetermined in the aetiology of T1D (89).

Evidence has suggested that the regulation of the gut immune system may be involved in the development of T1D (88). The role of Rotavirus, and rotavirus vaccination has been studied without consistent results (78), and recent studies have not been able to show any differences in T1D risks after Rotavirus vaccination (65, 90, 91).

2.3 COMORBIDITY OF COELIAC DISEASE AND TYPE 1 DIABETES

2.3.1 History

The coexistence of CD and T1D was first described in 1969 by John A Walker-Smith and W Grigor (92). They reported a short case study in a letter to the Editor of *The Lancet*, describing biopsy-proven CD in a girl with newly diagnosed T1D. Previously, Hooft et al had published a paper on a series of children with T1D and malabsorption (93), even though CD had not been confirmed by biopsies in all of them. More clinicians then started submitting case reports on CD in T1D (94-96).

Over the next three decades, research about these diseases, and the combination of them, intensified worldwide (97). From the 1970s onwards, several studies provided support for the concept that there was a causal relationship between CD and T1D (97-100).

The separate milestones in the history of each disease have provided a better understanding of the coexistence of CD and T1D. In the 1980s, HLA was described as a major genetic risk factor for CD (49) and this was later complemented by discoveries about sharing risk genes and protector genes (101). The genetic background, especially with regard to the HLA genes, has been the most well-studied factor in relation to the coexistence of these diseases (102).

In 2007, Lohi et al showed an increased prevalence of CD when the prevalence of T1D increased, implying a common denominator (98) (Figure 8).

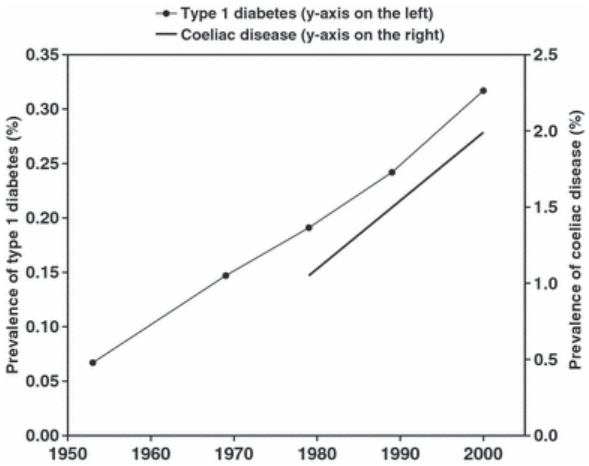


Figure 8. *Increasing prevalence in percentage (%) of both T1D and CD over time.*
Printed with permission from the publisher (98).

2.3.2 Guidelines

The publication of international recommendations about screening all children and adolescents with T1D for CD, improved awareness among the medical community (103), and may have led to an increased interest in the coexistence of both diseases.

Studies about the effects on the clinical course of diagnosing CD in children with T1D, together with other benefits, have driven the development of more guidelines (104). In addition, the clinical responses and benefits of treating CD with a gluten free diet in T1D patients had been reported (105, 106).

The 2004 diabetes clinical guidelines from the National Institute for Health and Clinical Excellence (NICE) recommended that individuals with T1D should be screened for CD at the time of diagnosis and then at least every three years (107, 108). However, NICE updated its guidance in 2009 to state that CD screening should only be performed at the time of T1D diagnosis (107, 109). It worth noticing that the latest version of NICE guidelines changed again and embraces the ESPGHAN guidelines for CD diagnosis in children (110).

Furthermore, the 2006-2007 ISPAD Clinical Practice Consensus Guidelines, about other complications and conditions associated with diabetes, stated that: *"Screening for coeliac disease should be carried out at the time of diagnosis and every second year thereafter. More frequent assessment is indicated if the clinical situation suggests the possibility of coeliac disease or the child has a first-degree relative with coeliac disease*" (111).

The latest version of the ISPAD recommendations were published as an update in 2018. They include recommendations about CD screening and also emphasise the importance of nutritional support if CD is diagnosed (112).

The 2012 ESPGHAN guidelines supported CD screening for patients with T1D, as well as for other conditions associated with CD (3). The 2020 ESPGHAN guidelines state that children with T1D could not be included in the no biopsy approach to CD diagnosis, due to lack of published data. However, they do encourage high-quality studies on children without symptoms, particularly those with T1D (22).

2.3.3 The case for screening

In modern medicine, principles of beneficence and ethical decisions based in evidence have been prioritized. To screen for a disease within these principles need to comprehend the same ethical criteria. The World Health Organization (WHO) criteria for screening from 1968 (113) fits well for the case of screening CD in patients with T1D (114).

The CD screening procedure that is common in clinical practice (115)follows the simplified algorithm showed in Figure 9.

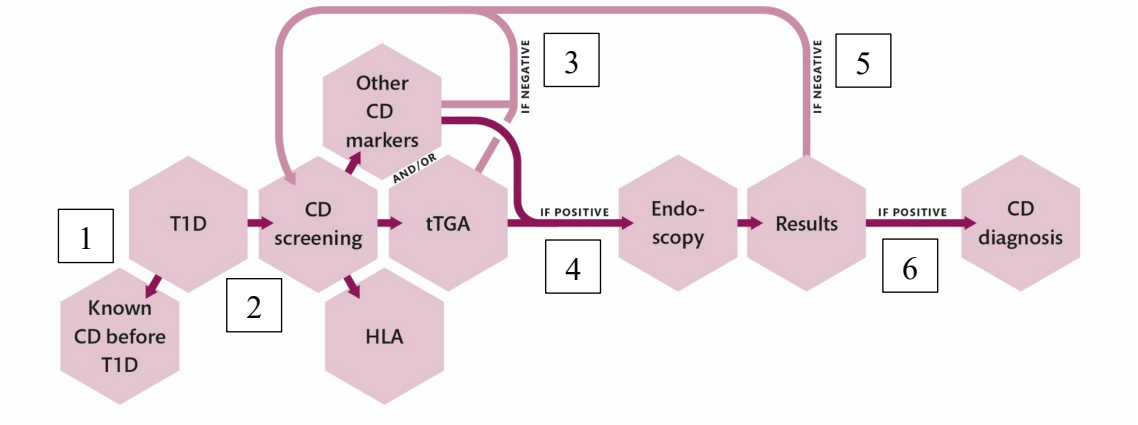


Figure 9. *Simplified algorithm for diagnosing CD by screening children and adolescents with T1D.*
1. Children and adolescents diagnosed with CD before their T1D diagnosis do not need to be screened.
2. The CD screening should include tTG antibodies, possible other biomarkers and a genetic HLA test.
3. If the CD biomarkers are negative, the screening should be repeated later. How often, and after how long, has not been decided.
4. If the CD biomarkers are positive, the child should be referred for an endoscopy to retrieve biopsies.
5. If the biopsy results are normal, the screening should be repeated later. How often, and after how long, has not been decided.
6. If the biopsy results show mucosal damage consistent with CD, the diagnosis can be given.

2.3.4 Screening prevalence

The prevalence of CD in T1D varies in different parts of the world. Though, results about biopsy proven CD in T1D are not yet available from all countries. In Figure 10, the prevalence of CD in different child populations with T1D diagnosis is presented.

In Sweden, the first study on the prevalence of CD in children with T1D showed that it was 21/459 (4.6%) (116). The next study, in 1999, showed a prevalence of 9/115 (7.8%) (117). Almost 10 years later, in 2008, a study was published that showed a prevalence of 29/300 (118), and another local study showed some years later a prevalence of 17/169 (10.1%) (119).

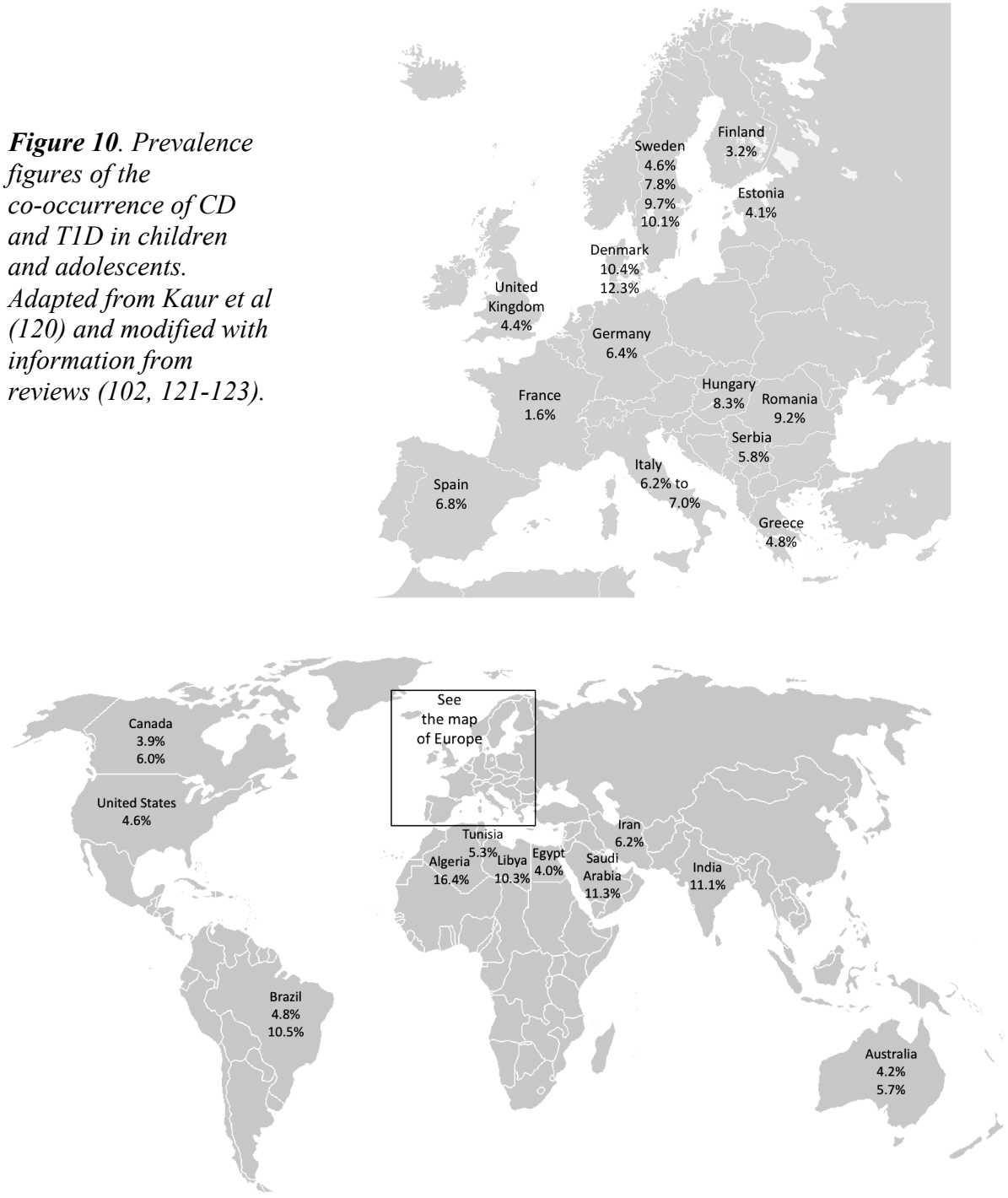


Figure 10. *Prevalence figures of the co-occurrence of CD and T1D in children and adolescents. Adapted from Kaur et al (120) and modified with information from reviews (102, 121-123).*

2.3.5 Immunological pathogenesis

Both CD and T1D have common features, including certain genetic risk factors and underlying mechanisms. Multiple triggers that start the immunological reaction that leads to the autoimmune response have been suggested (102). Similarities and differences about the knowledge on the pathogenesis of both diseases can be seen in Figure 11.

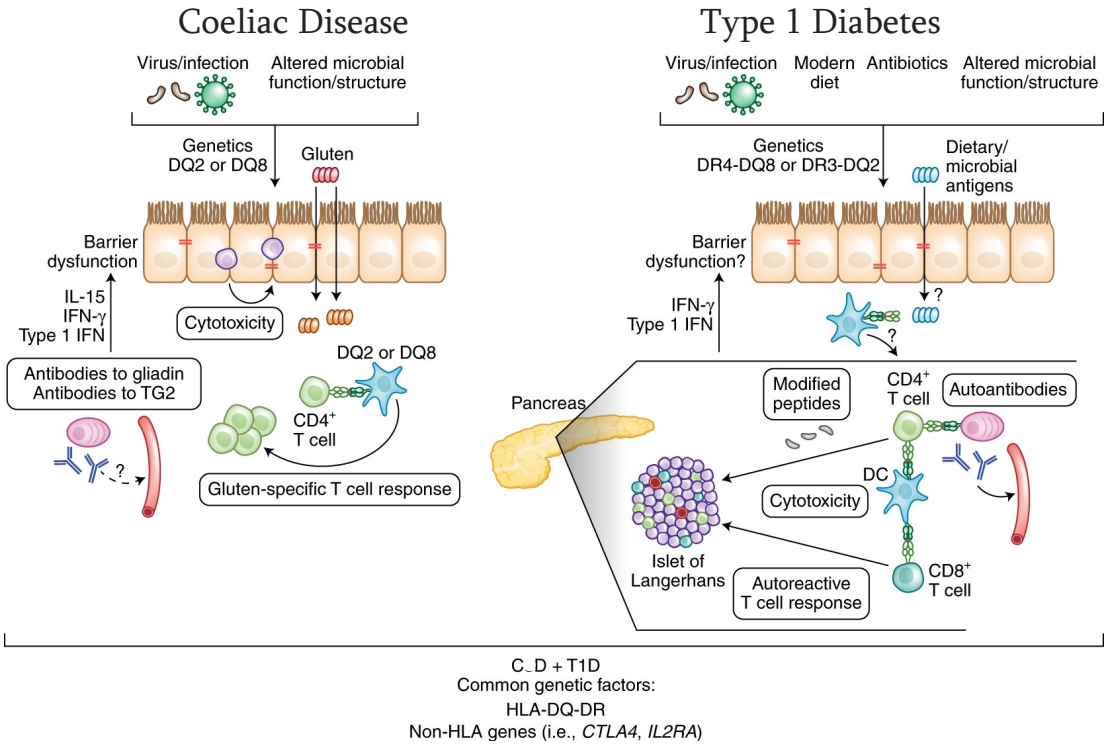


***Figure 11**. Model of common features of immunological pathogenesis in T1D and CD. Verdu et al state that: "In CD, bacterial or viral pathogens might alter the innate immune response or the gluten-specific T cell response, both of which are critical events for the full development of disease. In T1D, microbial products, viral infections, dietary practices and alterations in microbial structure and function have been suggested to be triggers of disease, but the mechanisms are less well understood."*
Printed with permission from the publisher (48).

2.3.6 Multifactorial aetiology

CD and T1D share genetics and may also share some environmental trigger factors (102). Gluten is the major trigger for CD and it has been suggested that it also plays a role in T1D, even if the possible mechanism that triggers T1D autoimmunity is not known (120). Perinatal risk factors may also affect the risk of the co-occurrence of T1D and CD (124). In addition, viral infections and disturbances in the gut microbiome and mucosal barrier function have been suggested as triggering factors (48), while early infections may have protecting effect (125) (Figure 11).

In the past decade, interesting prospective birth cohorts and population-based studies of populations at-risk for T1D and CD have begun. The results from these studies may help to identify common environmental risk factors and their mechanisms of action (48).

2.4 KNOWLEDGE GAP

When the first study in this thesis was designed, paediatric endocrinologists frequently expressed concerns about giving a second diagnosis to children and adolescents who had already been told they had T1D. In fact, the frequency of screening for CD in children and adolescents with T1D had been reported to be low (103). At the same time, our knowledge had increased about the potentially avoidable health consequences of undiagnosed CD (126-128).

Paediatric gastroenterologists in Stockholm were seeing many children and adolescents with T1D, but they were also noticing some delays in referrals for endoscopies and biopsies. We wanted to determine the real prevalence of CD at our clinic and to identify the difficulties in the procedure that children and adolescents with T1D were screened for CD. At that time, only a few studies had examined the prevalence of CD in Swedish children and adolescents with T1D (116, 117) and none of those studies had been conducted in Stockholm.

The Swedish CD epidemic had been thoroughly described by several studies (12, 18, 19, 129) before Study I, but no information had been published about the high-risk population of children and adolescents with T1D. When the results from Study I were published, they indicated that the CD epidemic had little effect on the prevalence of CD in birth cohorts with T1D in Stockholm. However, it was still unclear whether these results could be generalised to the whole paediatric population with T1D, which led to Study II.

Genetic factors shared by CD and T1D were already well known (130). However, the association between the CD biomarker tTG and diabetes autoantibodies, at the onset of T1D, had not been thoroughly explored. Therefore, this was the focus of Study III.

In 2012, ESPGHAN published new guidelines with different logarithms for diagnosing CD, included a specific algorithm that made a no biopsy approach possible in certain cases (3). However, children and adolescents with T1D were not included due to lack of data on asymptomatic children, and on children and adolescents with T1D (3).

Over the following years, several studies explored if the 2012 ESPGHAN guidelines could be applied to screened and/or asymptomatic children (37-42). Despite this, very few studies focused on children and adolescents with T1D (131). Therefore, when the latest 2020 ESPGHAN guidelines were published, they stated that children and adolescents with T1D were still excluded, due to the lack of data on the T1D population (22). This lack of information was addressed in Study IV, we wanted to explore whether it was safe to diagnose CD in children and adolescents with T1D without an invasive biopsy. That is why Study IV explored the CD biomarker tTG levels that would be needed to justify the no biopsy approach in children and adolescents with T1D.

3 AIMS AND HYPOTHESIS OF THE THESIS

The overall aim of this thesis was to expand current knowledge about CD in children and adolescents with T1D.

The specific aims were as follows:

- To investigate the prevalence of CD in children and adolescents with T1D, in Stockholm (Study I) and Sweden (Study II).
- To explore the prevalence of CD in children born during the Swedish CD epidemic, by comparing those born before (Study I) and after the epidemic (Studies I and II).
- To examine the association between HLA genotypes and the CD biomarker tTG levels and autoimmunity biomarkers for T1D in children and adolescents newly diagnosed with T1D (Study III).
- To show if it would be safe to diagnose CD without an invasive biopsy in children and adolescents with T1D, and, if that was the case, in what circumstances and with what level of CD biomarkers (Study IV).
- To assess whether the screening procedure for diagnosing CD in children and adolescents with T1D could be improved (Studies I, III, and IV).

The main hypotheses were as follows:

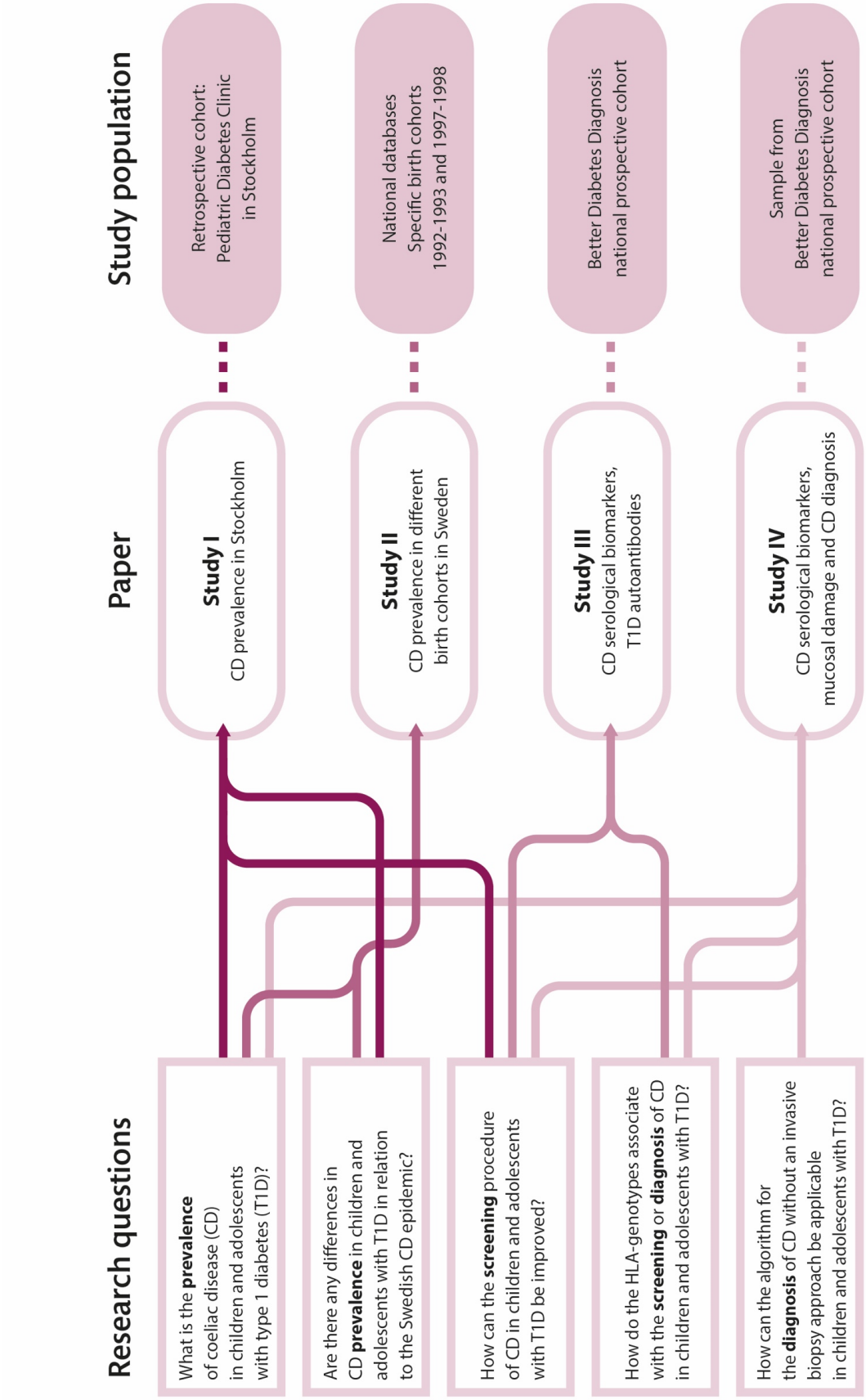
The main hypothesis for Study I was that the prevalence of CD in Stockholm in children and adolescents with T1D would be high in comparison to European countries. Another initial hypothesis was that the children with T1D would have a higher prevalence of CD during the Swedish CD epidemic, as the rest of the Swedish child population. A secondary hypothesis was that the number of adolescents lost to follow up would be high and the delay in referring children and adolescents for a CD diagnosis would be too long.

However, as the hypothesis about high prevalence during the Swedish CD epidemic was refuted in Study I, the hypothesis for Study II was that children and adolescents with T1D would not follow the same pattern as the general population and would have a similar prevalence of CD in birth cohorts born during and after the Swedish CD epidemic.

In Study III, the main hypothesis was that the CD biomarker tTG levels would be related to the HLA genotype, but not to the diabetes autoantibodies, IAA, GADA, IA-2A or ZnT8A, in children and adolescents with newly diagnosed T1D.

In Study IV, the hypothesis was that high levels of tTG, as a CD biomarker in peripheral blood, would be a reliably way to give CD diagnosis in children and adolescents with T1D, as in other screened or asymptomatic children. The hypothesis regarding improvement of screening procedure was that at least two-thirds of the children with T1D and with suspicious CD by screening could have avoided an invasive biopsy procedure to confirm CD.

4 OVERVIEW OF THE STUDIES



5 RESEARCH APPROACHES

5.1 STUDY POPULATIONS

All studies included in this thesis were based on Swedish cohorts. Sweden is a Scandinavian country and its population was 10,358,538 in July 2020, compared with 8,590,630 in December 1990. Although the population has grown in the last 30 years, the number of children under 18 has been stable, with small fluctuations (132) (Figure 12).

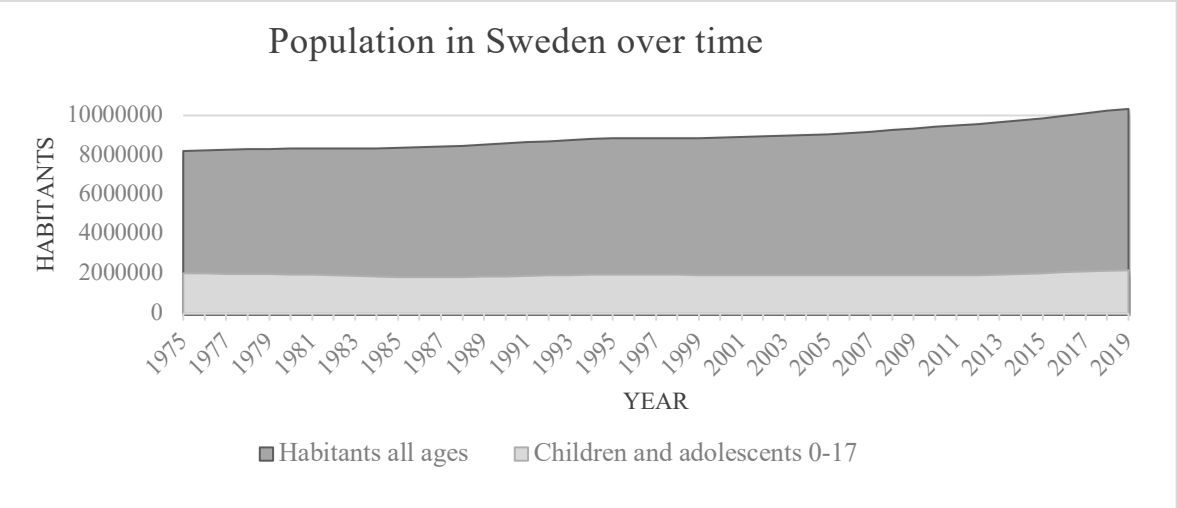


***Figure 12**. Population in Sweden, showing a stable proportion of individuals under 18 years of age (132).*

During the study periods covered by this thesis, all Swedish children with diabetes attended paediatric diabetes clinics in their local hospitals, at diagnosis and for follow-up appointments. All outpatient evaluations, hospital admissions and prescribed drugs were free of charge for patients up to the age of 18. In addition, gluten free products were subsidised for children and adolescents diagnosed with CD, but the upper age limit depended on which region they lived in.

The children and adolescents studied in this thesis were born between 1981 and 2010. The different study birth cohorts are presented in the lower panel of Figure 13, on the next page, and this follows the graphs of the incidence of T1D and CD in the general childhood population.

5.2 STOCKHOLM COHORT

Study I was performed in Stockholm, the capital of Sweden, by researchers based in the Karolinska University Hospital. The hospital covered the central and north of the city: there were around 250,000 children up to the age of 18 years in 1995 and around 275,000 in 2004. This equated to about 15% of the Swedish child population (132).

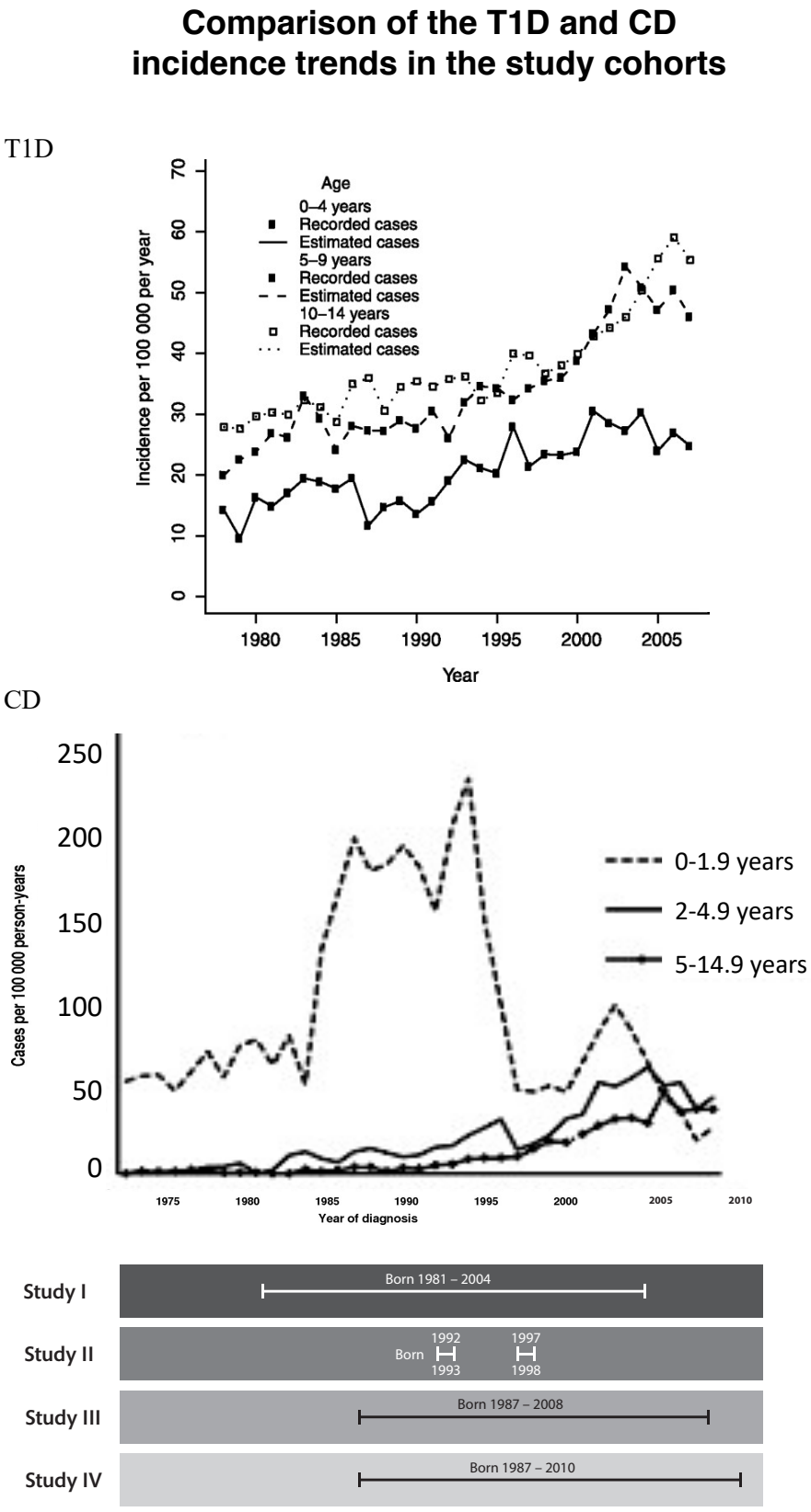


Figure 13. *Incidence of T1D (upper panel) and CD (middle panel) in Swedish children, according to age, and a timeline of the birth cohorts in Studies I-IV (lower panel). Notice the two different scales in the X-axes in the upper and middle panel. Graphs printed with kindly permission from Berhan et al and the publisher (73), and Namatuvo et al and the publisher (11) respectively.*

All children with T1D from birth to 17.9 years of age, who attended the paediatric diabetes clinic at St Göran Children's Hospital (1995-1997) and Astrid Lindgren Children's Hospital, a part of Karolinska (1998-2004), were registered in the DiaBase diabetes database. The WHO criteria for diagnosing diabetes were used (133), and 1,151 children in the DiaBase database from 1995 until 2004 were included in the retrospective analysis in Stockholm. The study cohort consisted of 847/1,151 of those subjects. Eight children had known CD before their diagnosis of T1D, three children underwent biopsy before screening due to symptoms and 836 children were screened. For comparison, we constructed three birth cohorts based on the years of the Swedish CD epidemic (18): before the epidemic (1984-1996), during the epidemic (1981-1983) and after the epidemic (1997-2004).

5.3 SWEDISH REGISTRIES

The Government-administered health registries in Sweden are the National Board of Health and Welfare. The information that they obtain incudes hospital-based inpatient and outpatient care (134).

Swedish Healthcare Quality Registries collect individual-based detailed clinical data, and provide an important source of information about specific diseases (135).

In Study II, children diagnosed with both T1D and CD were identified by merging information from Statistics Sweden and five national registries:

Statistics Sweden

Two birth cohorts from the general population were included in Study II: one cohort born in 1992-1993, during the Swedish CD epidemic and the other cohort born in the post-epidemic era of 1997-1998. Data on the population, sex, immigration and mortality were collected from Statistics Sweden.

Swedish inpatient, outpatient and day surgery registries

The inpatient, outpatient and the day surgery registries, which also are part of the National Board of Health and Welfare Register, were used to extract diagnostic data. The date when T1D was diagnosed was recorded, as well as the first visit with a CD diagnosis. The coverage and PPV for different diagnoses in these registries was assessed over time by an external review and validation study that showed that the included data were of a high standard (134).

Swediabkids and the National Diabetes Register

The disease specific Swedish Healthcare Quality Register Swediabkids, which covers patients below 18 years of age with diabetes, is a part of the National Diabetes Register (NDR) and provides a high coverage of individuals with T1D. The NDR was established in 1996 and Swediabkids started in 2000. Swediabkids comprises more that 7,000 children, and is nearly 100% complete (135).

5.4 BETTER DIABETES DIAGNOSIS STUDY

The BBD study is an ongoing nationwide prospective study with the aim to improve the knowledge of diabetes in Swedish children under the age of 18. The main aim of the study is to develop a more precise classification and diagnosis of diabetes, so that clinicians can provide the best treatment for each patient, and to increase knowledge on the underlying factors behind diabetes. The secondary aims include exploring co-morbidities and risk factors for late complications (136).

The study started in May 2005 and since then data on almost all children and adolescents with newly diagnosed diabetes in Sweden have been prospectively collected, including genetic analyses and autoantibody detection (136).

The American Diabetes Association criteria for classifying T1D have been used to determine the clinical diagnosis of diabetes in the BBD study (69). Furthermore, the diabetes diagnoses were re-evaluated after one year. All of the children who were included in Studies III and IV met the criteria for T1D.

The Study III cohort was a sub-study of 2,705 children and adolescents with T1D. They were subsequently recruited between May 2005 and November 2009, from 40/42 (95%) of the Swedish paediatric diabetes clinics.

Study IV included 2,035 children with T1D, recruited between May 2005 and December 2010, who were selected from 13 of the paediatric diabetes clinics. These centres collected results of anti-tTG and intestinal biopsies from patients investigated for CD. The 13 clinics that were involved in the study were: Göteborg, Helsingborg, Jönköping, Kristianstad, Linköping, Lund, Malmö, Norrköping, Stockholm, Västerås, Ystad, Örebro and Östersund.

5.5 COELIAC DISEASE BIOMARKERS

Several serological tests were used as CD biomarkers in Study I. IgA gliadin antibodies (AGA) were measured using an enzyme-linked immunosorbent assay (ELISA). The cut-off for AGA was <50 U/mL. In addition, IgA endomysial antibodies (EMA) were analysed using an immunofluorescence in-house technique with monkey oesophagus as the antigenic substrate. The cut-off titre for EMA was dilutions under 1:10. Last, but not least, IgA tTG were determined by ELISA (Binding Site, West Midlands, UK). The tTG cut-off was <4 U/mL (137). Prior to 2002, serological screening involved AGA and EMA. After 2002, tTG replaced EMA and AGA was analysed as a complementary test for children younger than two years of age. Furthermore, total IgA was checked to rule out IgA deficiency in all samples. When total IgA deficiency was found, the patients were tested with IgG endomysial antibodies.

The CD biomarker used in Study III was tTG. The tTG levels (kit No. L2KTD6) were analysed from serum samples on the Immulite 2000 analyser (Siemens Healthcare

Diagnostics, Deerfield, IL, USA), according to the manufacturer's instructions. Values > 50 IU were considered positive and values between 10 and 50 were considered borderline. In addition, the values were evenly distributed and there were no clusters of values.

Two different assays were used to analyse tTG levels in Study IV. Both assays were provided by Thermo Fisher Scientific systems (Legal Manufacture Phadia AB, Uppsala, Sweden). One was an enzyme-linked immuno-assay (EliA), the EliA Celikey IgA, with the level of positivity set at >10 U/mL. The other was an ELISA, the Celikey Tissue transglutaminase IgA Antibody Assay, with the level of positivity set at >8 U/mL. All children were screened for CD using anti-tTG when their diabetes was diagnosed and then at yearly intervals as part of the clinical routine. The autoantibody levels were grouped according to the last positive value before each patient's biopsy. Furthermore, total immunoglobulin A was tested to rule out immunodeficiency that would not detect anti-tTG of this type. Children with IgA deficiency were excluded in this study.

5.6 HLA TYPING

The HLA profile was analysed for all children included in the BDD study and the data were used in Studies III and IV. Blood samples were obtained at the clinical diagnosis of T1D and further processed by the Clinical Research Centre at Malmö, which is a part of Skåne University Hospital. HLA genotypes were analysed by sequence-specific oligonucleotide probes on dried blood spots and used directly for polymerase chain reaction amplification, as previously described (136, 138), using a DELFIA hybridization assay (PerkinElmer Inc., Waltham, Massachusetts, USA).

For comparison purposes, HLA genotyping were classified into four groups of genotypes, annotated with the short term nomenclature (50, 139): (i) DQ2/2, DQ2/X, and DQ2.2/X; (ii) DQ2/8; (iii) DQ8/8 and DQ8/X and (iv) DQX/X, where DQX was any haplotype other than DQ2, DQ2.2 and DQ8.

5.7 BIOPSIES

In Studies I and IV, the parents of the patients with positive serology were advised to let them have small intestine biopsies. The biopsies were obtained according to local clinical routines, mostly by endoscopy and sometimes by suction capsule. They were further assessed by local pathologists. In Study I, the examinations at the Department of Pathology, Karolinska University Hospital, were mainly performed by two pathologists. In Study IV, biopsies were assessed by the pathology departments of the different clinics. Further, in Study IV, the histological results were reviewed and scored by the same person, according to the revised Marsh-Oberhüber classification (26, 27) (Figure 3, page 8). In this context, it

is worth mentioning that biopsy evaluations from all the pathology departments in Sweden had been evaluated and had shown a high concordance with CD diagnoses (140).

5.8 STUDY DESIGN REGARDING SCREENING AND DIAGNOSIS

We have provided simplified schematic figures for each of the study designs. These show the screening procedure and the way we diagnosed CD in children and adolescents with T1D in the studies included in this thesis.

The majority of the children in Study I were screened for CD. There were also a small number who had already been diagnosed with CD and were not screened. One limitation of the study design was adolescents lost to follow up and diagnostic delays (Figure 14).

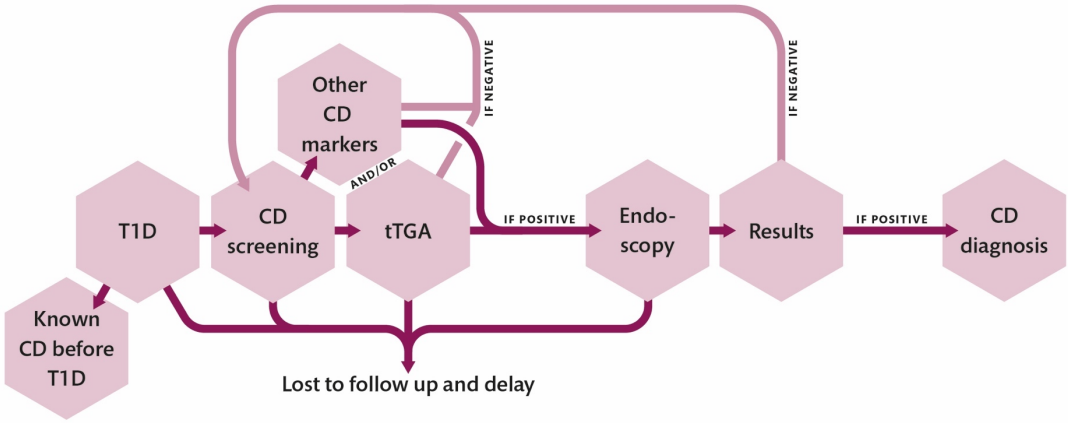


Figure 14. *Simplified algorithm for diagnosing CD by screening children and adolescents with T1D in Study I.*

Study II was a database cohort study in which two different birth cohorts were assessed and the CD diagnoses were retrieved from several databases (Figure 15).

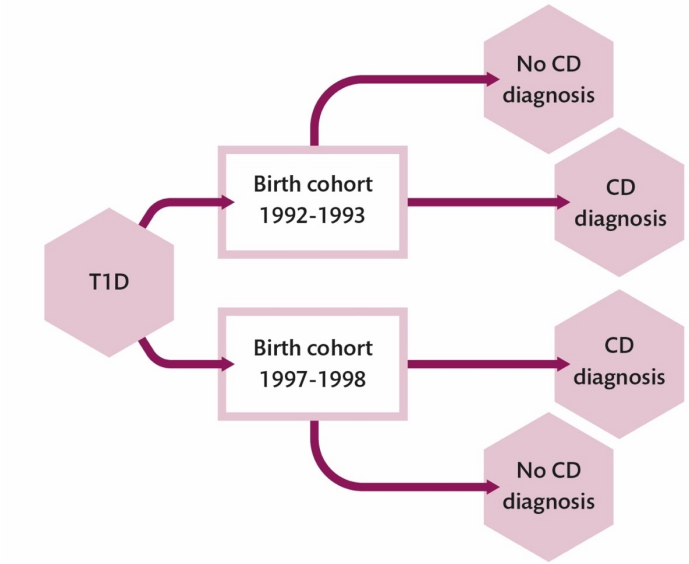


Figure 15. *Simplified algorithm for diagnosing CD in children and adolescents with T1D in Study II.*

In Study III, the endpoint was to assess CD autoimmunity and the value of HLA typing. T1D autoantibodies were also evaluated. In this study we did not have information about the children with known CD (Figure 16).

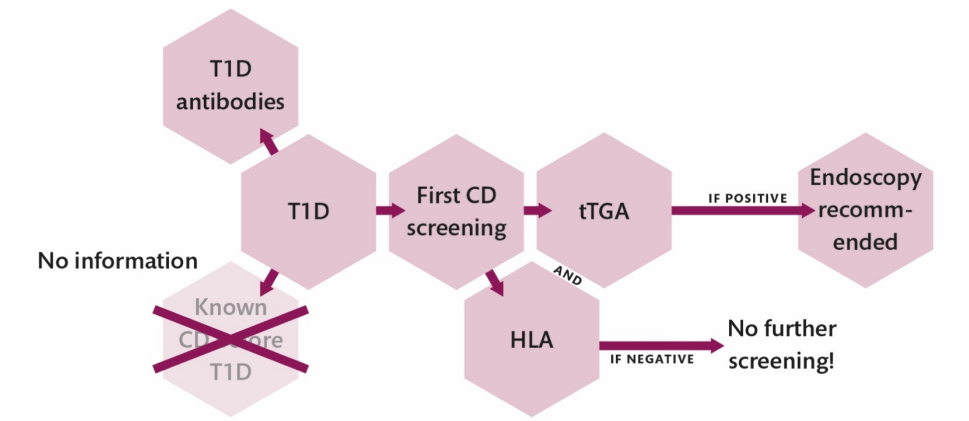


***Figure 16**. Simplified algorithm for CD screening in children and adolescents with T1D in Study III.*

Study IV comprised children with known CD before their T1D diagnosis. We assessed HLA typing and the levels of tTG autoantibodies compared with the mucosal damage seen in the biopsies (Figure 17).

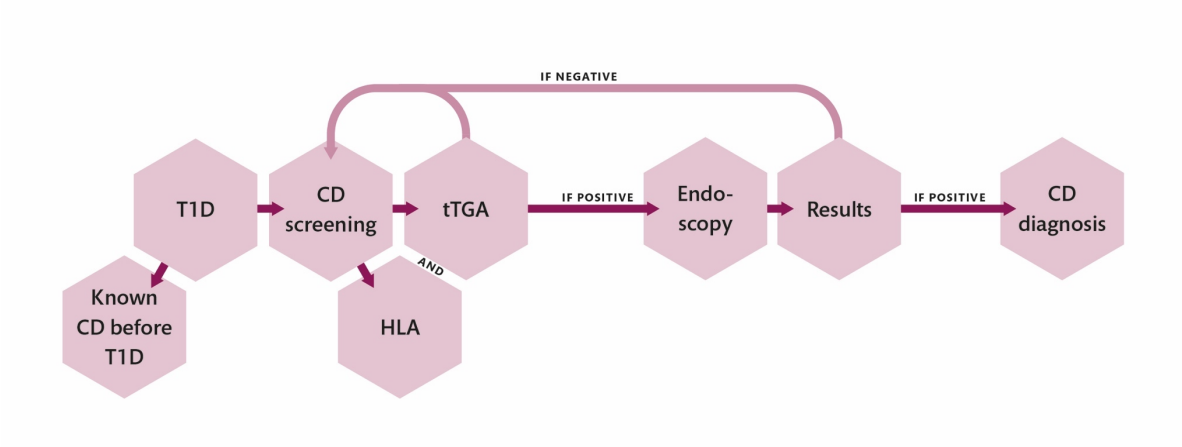


***Figure 17**. Simplified algorithm for diagnosing CD by screening children and adolescents with T1D in Study IV.*

Abbreviations in the algorithms:
CD, coeliac disease
T1D, type 1 diabetes
tTGA, tissue transglutaminase antibodies IgA
HLA, human leucocyte antigen

5.9 DIABETES AUTOANTIBODIES

GADA, IA-2, and IAA

Recombinant GADA and IA-2 were labelled with 35S-methionine (GE Healthcare Life Sciences, Amersham, UK) by in vitro coupled transcription and translation in the TNT SP6 coupled reticulocyte lysate system (Promega, Southampton, UK) as previously described (141). IAA were determined in a non-competitive radioligand-binding assay using 125I-insulin, as previously described (142). Details of the procedures, the intra-assay coefficients of the variations and the validation of the laboratory have previously been described (81). Samples were considered positive if GADA was > 50 U/mL, IA-2A was > 10 U/mL and IAA was > 1 RU. Furthermore, values for GADA of 35-50 U/mL, IA-2A of 6-10 U/mL and IAA between 0.81-1.0 RU were considered borderline.

Autoantibodies to Zinc transporter variants

The radioligand-binding assay for all three ZnT8A variants (ZnT8R, ZnT8W and ZnT8Q) were performed separately, as previously described (143), and the intra-assay coefficients of the variations and the results of the laboratory validation have also been previously described (81). The cut-off values for ZnT8RA were ≥75 U/mL, for ZnT8WA they were ≥75 U/mL and for ZnT8QA they were ≥100 U/mL to positive. Furthermore, values between 60-74 U/mL for ZnT8RA, 60-74 U/mL for ZnT8WA and between 70-99 U/mL for ZnT8QA were considered borderline.

5.10 STATISTICAL METHODS

Microsoft Excel and Microsoft Access were used for data handling (Microsoft Corp, Washington, USA). The data analysis was carried out using SAS system for Windows, version 9.1 (SAS Institute Inc, Cary, NC, USA) in Studies I and III, and SPSS software, version 25 (IBM Corp, New York, USA) was used in Studies II and IV.

The quantitative variables have been expressed as ranges, medians, means and standard deviations of the mean and the categorical variables have been described as frequencies and/or percentages.

All tests based on proportions were carried out using the test of homogeneity, based on the chi-square distribution or, in the case of small expected frequencies, Fisher's exact test. Comparisons between the three birth cohorts in Study I were carried out using analysis of variance, followed by a *post-hoc* test. The procedure proposed by Fisher was used to control for multiplicity.

The scatter plot in Study IV was created using GraphPad Prism 7.0 (GraphPad Software, California, USA).

In all studies, the 5% level of significance was considered. If there was a statistically significant result, the probability value (p-value) was given. When appropriate, the 95% confidence interval (CI) was presented.

5.11 ETHICAL APPROVAL

When we were planning the study designs for the papers in this thesis, there were six Regional Ethics Review Boards in Sweden under the Ministry of Education, which were located in Gothenburg, Linköping, Lund, Umeå, Uppsala and Stockholm. Today, since 2019, one central Ethics Review Authority archives all previous review requests.

The Regional Ethics Review Board in Stockholm approved Study I (registration number 2007/588-31/4). In addition, the BDD study was approved from the same regional board (2004/826/1) with amendments (2006/108-32/1, 2007/1383-32/1, 2009/1684/32 and 2011/1069/32), which regards Study III and the first part of Study IV.

The Regional Ethics Review Board in Lund approved Study II and the second part of Study IV (2014/476).

5.12 ETHICAL CONSIDERATIONS

All the studies were performed according to good practice for clinical investigations, based on the Declaration of Helsinki. The Declaration has been amended seven times since it was first published in1964 and the latest amendment was in 2013 (144).

Study I was a retrospective study and this meant that the children and adolescents and their families, could not be asked for written consent before reviewing their medical records. The Regional Ethics Review Board in Stockholm gave us permission to proceed with the study, because the knowledge we produced could benefit the study population, as one of the aims was to improve the screening procedures at the local paediatric diabetes clinic in North Stockholm.

Study II was based on medical data from different population-based registries and disease-specific Swedish healthcare quality registries. The National Board of Health and Welfare collects health information that does not require consent and it only provides data for studies that have received ethical approval. All the data were anonymized before we received it to protect patient privacy.

Studies III and IV were based on the same ongoing national prospective study, the BDD study (136). All the parents and capable children gave their informed, written consent to participate in the BDD study before inclusion. They were informed about the study design and the purpose of the study. In addition, they were informed that they could withdraw from the study at any time without any effect on their future treatment or care.

Furthermore, in Study III and IV, all the information about the HLA and autoantibody results was reported to the patient's local diabetes clinic. This directly benefitted the children who participated, as the information allowed clinicians to reach a more precise classification of their type of diabetes and assess their risk for co-morbidities, such as CD. The local paediatric diabetes clinic was then responsible for following up the patients.

In Study IV, we aimed to study if it was appropriate to diagnose CD in patients with T1D without a biopsy. A sub-population of children participating in the BDD study was selected and we collected follow-up information about the risk for CD, the development of CD biomarkers and the biopsy results. A separate ethical application for this part of the study was approved to retrieve data from the patients' medical records. The children that had already been diagnosed with CD will not benefit directly from the results of Study IV, but we hope the results will benefit children and adolescents with T1D towards a diagnosis of CD in the future.

6 RESULTS AND DISCUSSION

6.1 PREVALENCE OF COELIAC DISEASE IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

The prevalence of CD in children with T1D was calculated in three of the studies included in this thesis: Studies I, II and IV. CD autoimmunity was calculated as a proxy for CD in Study III.

Study I confirmed a high prevalence of CD in Swedish children and adolescents with T1D. The prevalence of CD in Stockholm was 77/847 (9.1%), with a 95% CI of 7.2-11.2% (Figure 18).

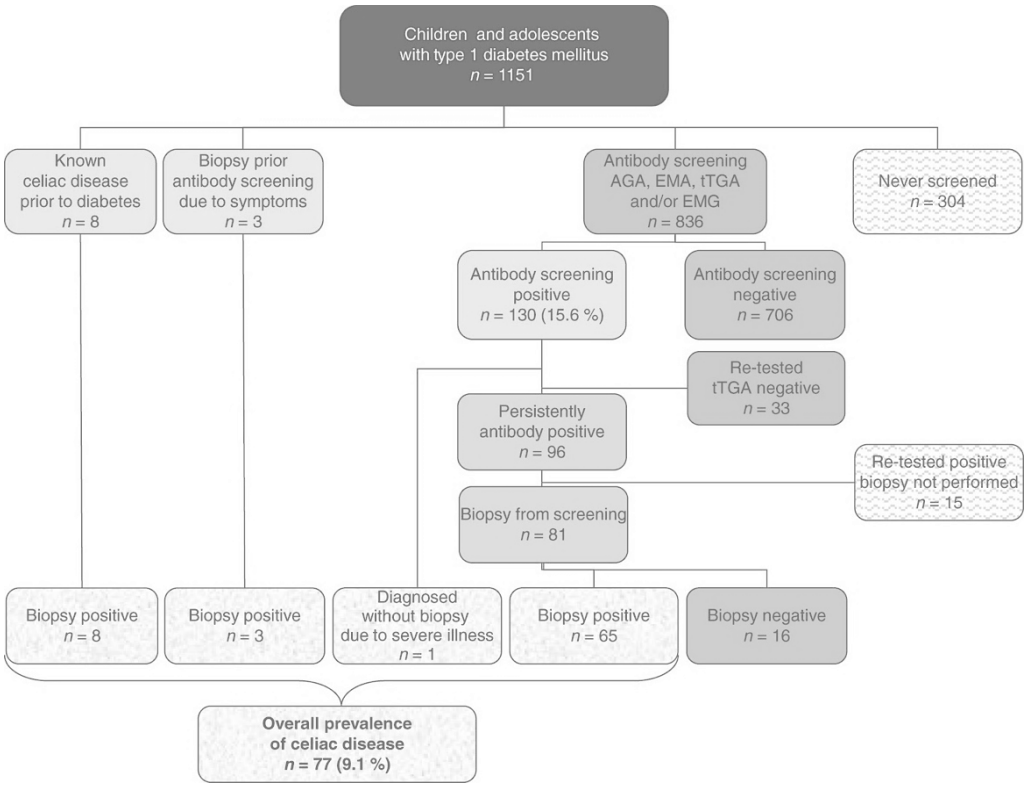


***Figure 18**. Flowchart of Study I.*
Printed with permission from the publisher (Study I).

The prevalence of CD in children and adolescents with T1D reported in Study I was higher than some previous Swedish studies (116, 117), but similar to others (118, 119). The 1999 paper by Carlsson et al (117) reported findings of an observational study that was performed in a tertiary hospital in Malmö, southern Sweden. The selection bias seen in that study, due to loss to follow up, was also a concern in our study when it came to the outcomes. The differences in CD prevalence between the studies in the 1990s and our higher prevalence in Study I, may be due to the study design, but a real increase in CD prevalence in the T1D population during this time cannot be excluded.

Study I confirmed similar high prevalence than in other parts of the country. Our results were similar to the findings in one study performed in Skåne, a province in southern Sweden (118), with a prevalence of CD of 29/300 (9.7%; 95% CI 6.6-13.6). In our Stockholm study the prevalence was slightly lower, but with a smaller 95% CI due to the larger T1D population that was screened. Nevertheless, the results showed to be very close, and these two studies used similar age range and birth cohorts, and may have some comparable limitations. In addition, confirming our results, a later study from Uppsala, a city situated north of Stockholm, showed a prevalence of CD slightly higher, 17/169 (10.1%), and within similar 95% CI (119).

Furthermore, the prevalence of CD found in Stockholm in children with T1D can be considered to be high worldwide (Figure 10). It was higher than many European studies and studies from Australia and North America (40, 122, 123). Whereas, at the same level as some other Scandinavian studies (105, 145), as well as studies from Libya (146), India (147) and in Saudi Arabia (148). However, not as high as the highest reported from a smaller study, with a CD prevalence of 19/116 (16.4%) in West Algeria (149). This variety in prevalence may be due to differences in study design and time of follow up, but the HLA-upset in the different diabetes populations may also have an impact of the prevalence of CD.

The strength of Study I was the possibility to study a screening procedure in a clinical setting, and also an inclusion of all the children with T1D in the North Stockholm area over a long period of time. In addition, the timeframe included birth cohorts before, during and after the Swedish CD epidemic. This provided us with the unique opportunity to study children with high-risk of CD in different circumstances.

One limitation of Study I, was the possible selection bias due to loss of follow up of some adolescents. We identified, on one hand, 15 children and adolescents that were very likely to have CD due to repeated high CD biomarkers, but were not referred for a biopsy procedure during childhood and, on the other hand, 304/1,151 individuals (26.4%) that were never screened during childhood. Even taking this into account, and assuming that no more cases of CD would have been found in these individuals, the prevalence of CD would still be high.

Similarly, the results from Study II confirmed that the national prevalence of CD was very high. The overall prevalence of CD in children and adolescents with T1D was 337/3,022 (11.1%; 95% CI 10.1-12.3). The two different birth cohorts that were created covered the period of the Swedish CD epidemic and one that covered the post-epidemic period. The prevalence od CD for the epidemic cohort was 176/1,642 (10.6%; 95% CI 9.2-12.2). This prevalence was not statistically different to the one showed in the post-epidemic cohort,1997-1998, with a prevalence of CD of 161/1,380 (11.7%; 95% CI 10.0-13.5) (Figure 19, on next page).

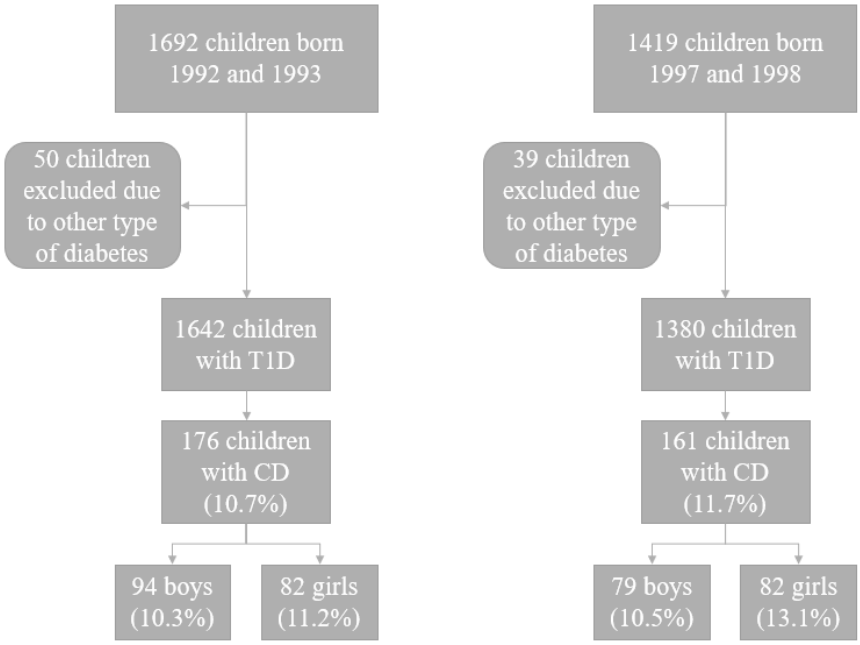


Figure 19. *Flowchart of study II. Selection of individuals diagnosed with T1D under the age of 17 years, one cohort born during the Swedish CD epidemic (1992-1993) and one born after the epidemic (1997-1998).*

In addition, the prevalence of CD in study IV was high, but not as high as in Studies I and II. (Figure 20).

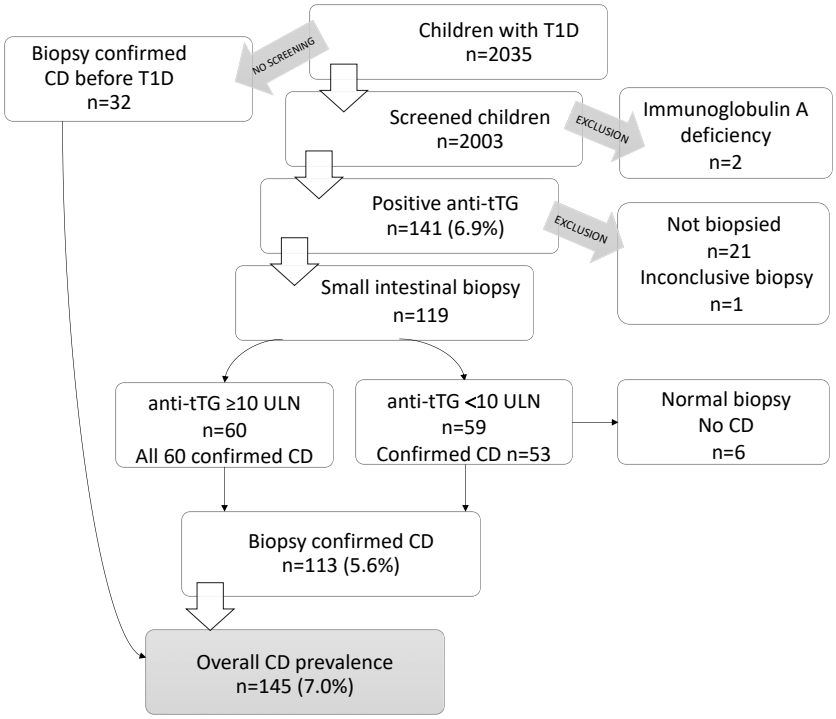


Figure 20. *Flowchart of study IV. The process for diagnosing CD in children with T1D with antibodies against tissue-transglutaminase (anti-tTG) and biopsies. The levels of tTG are presented as up to 10 times the upper limit of normal (<10x ULN) and at least 10 times this limit (≥10x ULN).*
Printed with the permission from Pediatric Diabetes.

Study IV was based on tTG IgA type. Positive tTG was found in 141/2,003 (6.9%) of the screened children with T1D, whereas CD was diagnosed in 113/2,003 (5.6%) of the screened children during the follow-up period, which ranged from eight to13 years from the diagnosis of T1D. When we added the 32 children who had been diagnosed with CD before T1D, the overall prevalence was 145/2,035 (7.0%; 95% CI 6.0-8.2).

Even though the overall prevalence of CD, seen in Figure 20, was high, it may have been underestimated due to the study design. There was some selection bias, as children with IgA deficiency were indirectly excluded because of the method that was used. A further 22 children were excluded: the parents of 21 children did not want them to have an endoscopy to obtain biopsies and one child had an inconclusive biopsy.

With regard to Study III, even when CD autoimmunity was the endpoint, the presence of tTG at the time of the T1D diagnosis was high at 148/2,705 (5.4%). These results about CD autoimmunity may suggest a high prevalence of CD already at T1D diagnosis (Figure 21).

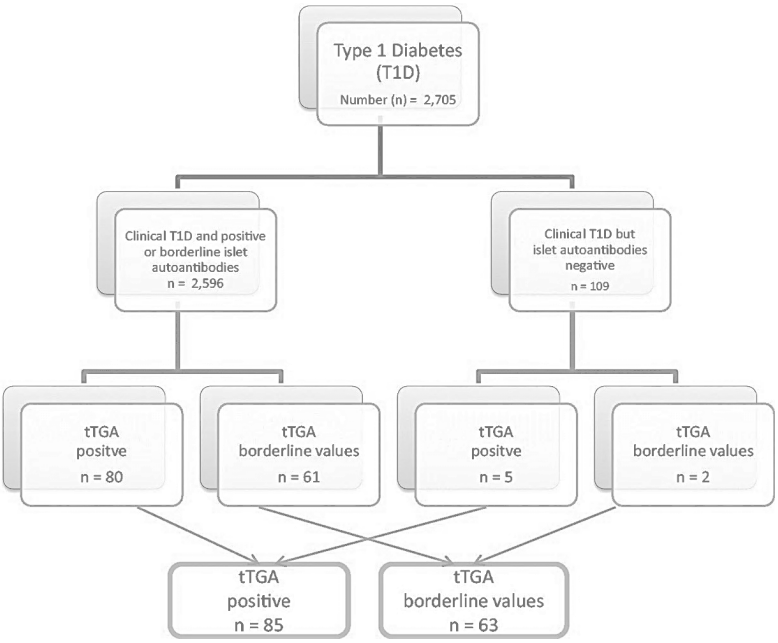


Figure 21. *Flowchart of Study III: the BDD study and tTG IgA type (here abbreviated tTGA) in children and adolescents newly diagnosed with T1D.*
Printed with permission from the publisher (Study III).

This 5.4% level of CD autoimmunity was also considered a high level for the first screening of CD in children and adolescents with T1D. However, we are aware that the prevalence of CD at the time of a T1D diagnosis could not only be based on CD autoimmunity and, furthermore, we did not have data on which children had CD diagnosed before they were diagnosed with T1D.

The results presented in this thesis, especially the early preliminary results from the first study, had an impact on how the Swedish endocrinology paediatric community followed the screening recommendations. When the first preliminary results from Study I were presented

to colleagues at our clinic in Stockholm, there were no national guidelines or international consensus about how often to screen children with T1D or for what period of time following their T1D diagnosis. Even when the local guidelines were in place at our Stockholm clinic, the recommendations were not always followed.

Several arguments have been used to validate repeated serological tests. That is maybe why endoscopies and biopsies have been postponed which have delayed the diagnoses of CD in children with T1D. One reason for this could have been some misconceptions about the need to start a gluten-free diet when children screened positive for CD. Another concern could have been the concept that the presence of tTG at the time of T1D diagnosis, was only a part of a general autoimmune reaction, and they would therefore disappear later. In addition to this, the guidelines from ISPAD did not recommended CD screening until 2007 (111). The findings from Study I helped convince the paediatric diabetologists in our clinic about the importance of following the local recommendations.

By the time the results from Study I were presented, the guidelines did not specify how often children with T1D should be screened for CD or for how long the screening should continue. Furthermore, not all other guidelines followed ISPAD's lead and recommended that T1D patients were screened for CD (150). In addition, the ESPGHAN guidelines from 2012 recommend retesting at-risk children at intervals, but with no firm evidence of frequency (3), and the 2020 ESPGHAN guidelines had no additive information about it (22).

6.2 THE SWEDISH EPIDEMIC OF COELIAC DISEASE IN TYPE 1 DIABETES

Studies I and II assessed the *de novo* knowledge about children and adolescents with T1D who developed CD in unique, paediatric Swedish populations before (only Study I), during and after the country's coeliac epidemic.

The aim of Study I was to assess the overall prevalence of CD in children and adolescents with T1D in Stockholm, as well as to determinate the prevalence of CD in birth cohorts before, during and after the Swedish CD epidemic. There were some epidemiological restrictions with regard to the birth cohorts born in Stockholm in 1981-1983 (pre-epidemic), 1984-1996 (during epidemic) and 1997-2004 (post-epidemic), such as the screening frequency and duration. However, there were no statistically significant differences in the prevalence of CD in these cohorts (Table 3).

***Table 3**. CD in children and adolescents with T1D in relation to the Swedish CD epidemic. The prevalence in each year range is presented as the percentage and 95% CI. Printed with permission from the publisher (Study I).*

Birth cohort	Number of CD cases before screening	Number of CD cases found by screening	Total number of CD cases	Mean CD cases per year of birth	Number of screened children	Prevalence in each cohort (%) (95 % CI)
1981-1983	2	3	5	1.67	126	3.9 (0.6-7.3)
1984-1996	8	52	60	4.62	630	9.4 (7.1-11.7)
1997-2004	1	11	12	1.5	80	14.8 (7.1-22.5)

One noticeable limitation with regard to the external validation of the results in Study I was the imbalance in the number of screened children with T1D between the birth cohorts. Another limitation was the follow-up period, which was especially short in the children born after the epidemic and even shorter in the children born during the new Millennium.

Importantly, the results in Study I encouraged us to explore the prevalence of CD in paediatric patients with T1D at a national level. Study II was designed to identify the prevalence of CD in T1D patients in two separate birth cohorts, one born during the epidemic and one born post-epidemic. However, the assessments of the 1981-1983 pre-epidemic cohorts were not included. This was because the national data about people born in the 1980s was considered less reliable than later records.

The prevalence of CD in these birth cohorts during and after the epidemic was similar and confirmed the findings of Study I: 176/1,642 (10.7%; 95 % CI 9.2-12.2) individuals with T1D born 1992-1993 were diagnosed with CD during childhood, compared to 161/1,380 (11.7%; 95% CI 10.0-13.5) individuals with T1D born in 1997-1998 (Figure 19). These two-year periods were chosen from the 1984-1996 epidemic and 1997-2004 post-epidemic birth cohorts, to be representative and to give an appropriate power, with a total target population of 240,844 individuals born in 1992-1993 and 179,530 individuals born in 1997-1998.

The main strength of Study II lay in its design, as it was a national assessment of the whole population of individuals with T1D in two birth cohorts, one during the epidemic and one post-epidemic, which were comparable in size and follow-up periods. The diagnosis of both T1D and CD during childhood was assessed in an identical way, which minimised the risk of selection bias, and possible regional differences could not affect the general results.

It would be unethical to replicate the environmental changes that took place during the Swedish CD epidemic. Therefore, to being able to study this natural occurrence by using various data sources provided us with a unique opportunity. It may be argued that databases are not always complete, but to our knowledge the Swediabkids register within the NDR have been reported to have achieved almost 100% coverage of all children diagnosed with diabetes. Moreover, the NPR has been validated and this showed a very high PPV for CD diagnosis from 2001 (134).

One possible limitation of Study II was that we did not register breastfeeding information or whether the parents did, or did not, follow the current feeding recommendations for gluten when the study was carried out. Another possible concern was the potential bias of misclassified diagnoses. We restricted the cohort to T1D, by checking for a T1D diagnosis in the Swediabkids and NDR. The children and adolescents diagnosed with another type of diabetes than T1D in Swediabkids, including maturity onset diabetes of the young and secondary diabetes, were not included, even if they had a T1D diagnosis in the NPR, because not all different types of diabetes have an individual classification in this register as it is in Swediabkids. This suggested that we did not have a significant misclassification bias for diabetes. In contrast, due to the study design, misclassification of CD diagnoses may have

occurred, because we did not have the chance to evaluate the results of biopsies or serology tests. However, the accuracy of CD cases that have been diagnosed by biopsies has been validated by Ludvigsson et al, with regard to all pathology centres in Sweden (140). Furthermore, the overall CD prevalence, was in the same range as in the study from southern Sweden (118), from Upsala (119) and Study I, pointing towards a good accuracy of the results.

To summarise, the results produced by Studies I and II about the CD prevalence rates during the Swedish CD epidemic indicated that children with T1D, who have a high risk of developing CD, were not affected by changes in environmental factors in the same way as these changes affected the general paediatric population.

These findings suggest that the genetic importance was superior to the environmental changes. Furthermore, they may indicate the need for observational and interventional studies to be revaluated and that studies conducted in children and adolescents who have high-risk genes for T1D (60, 151-153) may implicate low external validity for the whole paediatric population. The same may be true for other at-risk populations for CD (58, 59, 61). As Ludvigsson and Lebwohl commented in an editorial (154), it may not be advisable to base new paediatric feeding recommendations for CD based on studies that have only focused on a specific group of children, such as babies at-risk for T1D. The results of Studies I and II support this editorial. They suggest that it may not be wise to have feeding recommendations based on a population at-risk for T1D, which is in he line with our findings where the population that developed T1D did not appear to be clearly influenced by the environmental changes in infant feeding that gave rise to the Swedish CD epidemic in the general population. It would be interesting to evaluate if differences in HLA genotypes, and variants within a given genotype (155, 156), have different affects in the T1D population. In addition, studies that use a similar design to ours could be carried out to see if first-degree relatives of individuals with CD had the same prevalence of CD during and after the epidemic.

6.3 HLA GENOTYPES IN RELATION TO BIOMARKERS FOR COELIAC DISEASE, DIAGNOSIS OF COELIAC DISEASE AND AUTOIMMUNITY IN TYPE 1 DIABETES

The aim of Study III was to evaluate genetic associations between different HLA genotypes and the CD biomarker tTG, as well as T1D islet autoantibodies. The study was based on data from the nationwide prospective cohort of newly diagnosed children and adolescents with T1D in Sweden, the BDD study. HLA-DQ2 and/or HLA-DQ8 had been shown to be present in 92% of the children with T1D in this cohort (80, 81). We wanted to study if positive tTG results were associated with a special HLA genotype and if there were any relationship with the spectrum of T1D autoantibodies in these patients. We explored tTG levels at the time of T1D diagnosis and compared them to the HLA genotype and the T1D autoimmunity markers, namely GADA, IA-2A, IAA and the three variants of ZnT8A, all measured at the time of the T1D diagnosis.

The presence of HLA-DQ2 and/or DQ8 were found in 91% of the patients, whereas around 40% had the high-risk genotype for T1D, DQ2/DQ8, the rest of the population (8%) had other genotypes and 1% were unclassified.

In Study III, HLA-DQ2/2 was the highest risk genotype when tTG was present, followed by DQ2/DQ8 and DQ2 in combination with other HLA haplotypes. However, most children with positive tTG had the genotype DQ2/DQ8, but this was also most frequent in the T1D population. Our findings agreed with other studies exploring HLA and confirmed CD in children with T1D. Our findings that HLA-DQ2/2 was the high-risk genotype also agreed with other studies that evaluated the prevalence of CD in high-risk populations (10, 157) (Table 4).

***Table 4**. The distribution of HLA genotypes in 2,671children with newly diagnosed T1D, and the relationship with tTG.*
Printed with permission from the publisher (Study III)

HLA genetic markers	**tTG positive** ***n* (%)**	**tTG borderline values** ***n* (%)**	**tTG positive and borderline values** ***n* (%)**	**tTG negative** ***n* (%)**
DQ2/2, DQ2/X and DQ2.2/X n=503	22 (4.4)	17 (3.4)	39 (7.8)┼	464 (92.2)
DQ2/2 n=172	*10 (5.8)*	*8 (4.7)*	***18 (10.5)***	*154 (89.5)*
DQ2/X n=284	*12 (4.2)*	*8 (2.8)*	***20 (7.0)***	*264 (93.0)*
DQ2.2/X n=47	*0 (0)*	*1 (2.1)*	***1 (2.1)***	*46 (97.9)*
DQ2/8 n=787	41 (5.2)	22 (2.8)	63 (8.0)	724 (92.0)
DQ8/8 and DQ8/X n=1,165	21 (1.8)	22 (1.9)	43 (3.7)┼	1,122 (96.3)
DQX/X n=216	0 (0)	0 (0)	0 (0)	216 (100)

n denotes number; % denotes percentage
Nomenclature (50, 139):
DQ2 denotes (DQA1*05:01-DQB1*02:01); DQ8 denotes (DQA1*03:01-DQB1*03:02);
DQ2.2 denotes (DQA1*02:01-DQB1*02:01); DQX is another haplotype that DQ2, DQ2.2 and DQ8
┼ *p*-value 0.00001 (DQ2/2, DQ2.2/X and DQ2/8 compare to DQ8/8 and DQ8/X)

The presence of HLA-DQ2 showed a greater statistically significant difference than the other haplotypes in this T1D population, including DQ8. Furthermore, we found that the only child that did not have HLA-D2.5 or DQ8, had the HLA-DQ2.2 variant, which also confers a risk for CD (50, 139, 158).

To our knowledge, this was the first study to investigate the association between HLA, tTG and diabetes autoantibodies at the diagnosis of T1D, including the three variants of ZnT8A.

Even though we did not find any association between these T1D autoantibodies, this prospective study provided us with a unique possibility to explore if different T1D autoantibodies could predict the presence of tTG at T1D diagnosis.

The major strength of this study was the large, national, population-based cohort, including virtually all of the Swedish children and adolescents with newly diagnosed T1D during the study period. Another strength was that HLA, autoantibodies and tTG analysis were performed by the same laboratory, under the same conditions during this study. The missing data on HLA, which was 34/2,704 (1.3%) of the children and adolescents studied, was very low compared to other studies in Europe (39) and in USA (159).

One major concern about Study III was the possibility of transient autoantibodies. Previous papers have discussed that low ranges of tTG can revert to normal over time after T1D diagnosis. However, the few studies that formed the basis of ongoing discussions did not conduct biopsies to rule out CD in all the children with elevated tTG, or classified all these children as potential CD, as recommended by the Oslo classification (5). Using a clinical and serological follow up, without performing a biopsy in all the children with low tTG, may also be a major limitation of the studies that reported transient low tTG (160-163). We welcome new studies on the subject about transient or fluctuating tTG, but this matter did not affect how we assessed the results in Study III, as the endpoint was to identify differences in the autoimmunity load at T1D diagnosis.

HLA genotyping was also analysed in Study IV. In this study we were able to determine the HLA genotype in children and adolescents with T1D, with the endpoint of CD. The most frequent genotype was again HLA-DQ2/DQ8 (42.4%) followed by DQ8/DQX (21.1%). The highest risk haplotype was DQ2, as in Study III.

All but one of the investigated children and adolescents with T1D and CD had the HLA high-risk alleles for CD: HLA-DQ2 and/or -DQ8. The child that did not have those high-risk alleles, had Down Syndrome and this patient's HLA genotype was DQ7/DQ9. Both DQ7 and DQ9 have been shown as a risk for CD. DQ7 has been related to be the most frequent HLA in the very few CD patients in the general population without DQ2 or DQ8 (164, 165). In addition, DQ9 has also been showed to confer a risk for CD (166).

To summarize the results about HLA, the high-risk HLA genotypes DQ2 and/or DQ8 were virtually always present in children and adolescents with T1D and CD autoimmunity or diagnosed with CD. These findings suggested that, for the purpose of screening, the role of HLA typing is limited, and could be reserved to identify the approximately 8% of the children and adolescents with T1D that would not be at-risk for developing CD. These individuals could avoid further CD screening.

6.4 TISSUE TRANSGLUTAMINASE ANTIBODIES LEVELS AND BIOPSY RESULTS

The most important result of Study IV was that it confirmed that high levels of IgA tTG could predict CD in children and adolescents with T1D. When tTG was 10 times above the ULN, the mucosal damage in paediatric patients with T1D was predicted with high certainty to have CD. Study IV was based on a national longitudinal population-based prospective study, including a large cohort of paediatric patients with T1D who were routinely screened for CD (Figure 20). These results were similar to the results from longitudinal large studies in children screened for CD for various reasons, not just T1D (37, 41, 42).

It is interesting to note that also tTG levels that were seven times above ULN provided an accurate indication for a CD diagnosis. These results disagreed with a recent study from the Netherlands (167), which suggested that tTG levels of 11 times ULN and above were accurate for CD diagnosis. The major difference between this Dutch study and our Study IV was that six different kits were used during the study period in the Netherlands, while we only used two kits from the same manufacturer.

Only a few previous studies have exclusively reported on the implications of the ESPGHAN 2012 guidelines for children and adolescents with T1D. In addition, we are only aware of one study about CD diagnosis in paediatric patients with T1D that discussed the ESPGHAN 2020 guidelines (167). Table 5 shows a comparison of the methodology and the results of our study in comparison with previous studies.

Table 5. *Comparison of results from four different studies (columns 2-5) (131, 167-169) and our Study IV (column 6) regarding levels of tTG and biopsy proven CD in children and adolescents with T1D.*

PUBLICATION	Popp et al, 2012	Joshi et al, 2019	Puñales et al, 2019	Wesselts et al, 2020	Study IV
Study design	Consecutive cohort, cross-sectional test	Longitudinal, population-based diabetes register, retrospective analysis	Random sample, crossectional test	Retrospective observation of biopsied children	Longitudinal, nationwide population-based study, and retrospective analysis of medical records
Setting	Unclear	Database serach, one centre	Diabetes reference center	Multicentre (13 centers)	Multicentre (13 centers)
Country	Finland &Romania	Western Australia	Rio grande do Sul, Brazil	Netherlands	Sweden
T1D population	181	936	881	Unknown	2003
Age	0-18	0-17.9	0-21	0-18.9	0-17.9
Follow-up time	NA, median time from T1D diagnosis aprox 3.5 years	NA	NA	NA	range from 8-13 years
CD biomarkers	tTG and EMA	tTG	tTG	tTG	tTG
Biomarkers' methodology	ELISA	EliA, Phadia 250, Thermo Fisher	Enzime immunoassay, Eurospital	Six different ELISA manufacturers	ELISA, ELiA, both from Thermo Fisher Scientific systems, Legal Manufacture Phadia AB, Uppsala, Sweden
Biopsy evaluation	Marsh-Obenhuber	Unknown	Marsh-Obenhuber	Marsh-Obenhuber	Marsh-Obenhuber
Number of biopsies	11	66	62	63	119
CD (%)	9 (5.0)	66 (7.1)	49 (5.6)	NA, CD confirmed in 52 children	113 (5.6)
x ULN	non specified	11x ULN and above	only intermediate and x1 ULN	11x ULN and above	10x ULN and above
Number of proven CD in high-titre tTG out the number of biopsies	7 out of 7	35/35	NA / only gradients of tTG were described	47/50 for above 11x ULN, and 47/51 for above 10x ULN	60/60

Abbreviations: EMA, endomysial autoantibody, ELISA, enzyme-linked immunosorbent assay; EliA, enzyme-linked immunoassay, ULN, upper limit of normal, NA not applicable.

The main strength of Study IV was the prospective multi-centre data collection, based on the longitudinal, population based BDD register. The BDD study register included a large number of participants and had very high national coverage (136). As shown in Table 5, the number of children that were screened and the number of biopsies that were performed were higher than previous studies. The data analysis was retrospective, but the BDD study design implicated no considerable selection bias, as we found few exclusions, missing data or loss to follow-up.

Another strength of the study was the association between tTG levels, and the mucosal damage observed in the biopsy samples (Figure 22), assessed with only two different biomarker tests (ELISA and EliA), from the same manufacturer. A common concern about validating tTG is whether the test kits are universally available. The EliA kit, that was one of the tests used in our study, is currently universally available and the reported accuracy has been very high (170, 171).

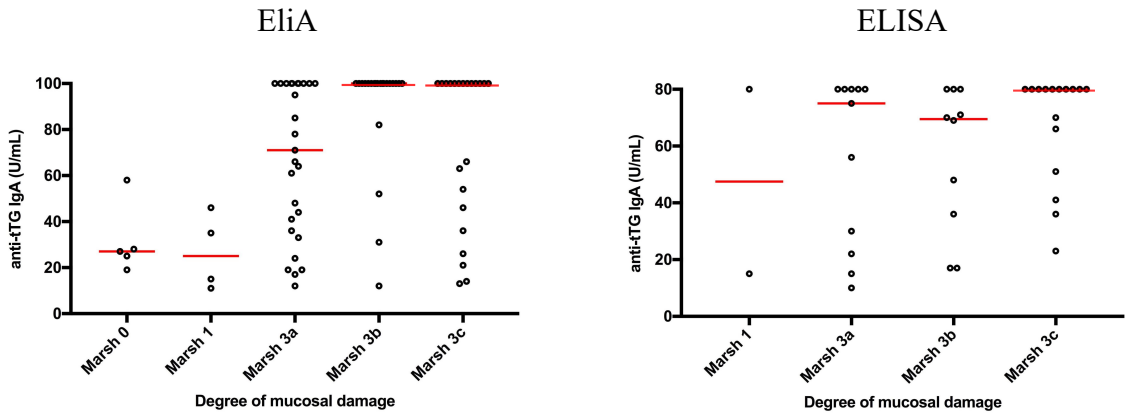


Figure 22. *Degrees of mucosal damage in relation to anti-tTG using the EliA method to the left and the ELISA method to the right. The lines represent the median. The levels of anti-tTG correlated to the degree of mucosal damage.*
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A possible limitation of the study design was that the biopsies were assessed and classified by the local pathologists from each clinic and that the answers were then validated according to the Marsh-Oberhüber scale by a single non-blinded co-author. However, we based the study design on the notion that a previous validation study of biopsies, that covered all of Sweden's 28 pathology departments, concluded that it is feasible to identify CD using regional biopsy data (140). This validation study also emphasized that the specificity of the CD biopsy results was particularly high in biopsies with villous atrophy (140).

One restriction of Study IV was that we did not particularly account for patients with IgA deficiency. Since ELISA and EliA tests are IgA based, all the children were tested for IgA deficiency at the time of the screening. If they were reported as IgA deficient, the method used to determine a CD diagnosis was different, as the ESPGHAN and the United Kingdom guidelines recommend that children with IgA deficiency are always recommended to undergo a biopsy (22, 36).

Another concern about autoantibodies would be that we did not test for endomysial autoantibodies (EMA). Both the ESPGHAN 2012 (3) and ESPGHAN 2020 guidelines (22) recommend EMA as a second test. In the latter, studies on EMA accuracy were summarized and showed a higher sensitivity, but a lower specificity than tTG (22). We did not include EMA in our study as local measurements were not available in all parts of Sweden. However, we checked for EMA levels in a sample of children from the South of Sweden, where the test was available, and found a good correlation between tTG levels and EMA results (Table 6).

Case number	tTG	EMA level 1:	Marsh-Oberhüber
1	19	400	3C
2	110	1600	3B
3	114	1600	3C
4	141	400	3A
5	159	1600	3C
6	162	1600	3A
7	>200	1600	3B
8	>200	1600	3B
9	>200	1600	3C

***Table 6**. Children that were tested with EMA as a second test after positive anti-tTG and the correlation with the Marsh-Oberhüber classification of biopsies. EMA levels were considered normal if dilutions were under 1:10 and positive in dilutions greater than 1:10. The maximum level in the test was 1:1600.*

These results for tTG and EMA in relation to high tTG levels were consistent with previous studies (170, 171). In a recent Spanish study, Donat el al retrospectively examined discordant autoantibody results where the tTG and EMA findings were different. They found that only three children with T1D with a normal mucosa were EMA negative and had low levels of tTG (172).

Furthermore, a common discussion concerning CD biomarkers in children and adolescents with T1D, has been concerns about transients CD autoantibodies. However, as mentioned before, there are few studies regarding transient or fluctuating serological autoantibodies in the T1D population (160, 163, 173). Rinawi et al concluded that children with "slightly" elevated anti-tTG should be followed up when they were on a diet containing gluten (173). Importantly, in the study from Waisbourd-Zinman et al, the levels of tTG were statistically significant lower in the group of children with normalized tTG levels compare to those with CD diagnosed with biopsies (163).

Nonetheless, Study IV had focused on children with high anti-tTG antibodies, and we could not find any study that had reported consistent findings about transient high levels of tTG. Moreover, a population with very high markers for CD may be different when compared to children with low antibody responses, as suggested by Mubarak et al (174).

To summarize, we found a good correlation between tTG levels and mucosal damage and that tTG that was above 10 times the ULN could predict CD. As we found that 60/119 children had tTG that was more than 10 times above the ULN, it would be possible to diagnose CD without invasive biopsies in about half of the children with T1D and elevated tTG.

6.5 REFLECTIONS ON SEX DIFFERENCES

Overall, we found some differences between females and males in our studies. These differences can be due to the interaction between genetic, epigenetic and environmental factors, in the influence of sex hormones. The reasons for differences in the prevalence of autoimmune diseases in females and males are still mostly unknown (9). The possible influence of sex hormones and chromosomes on the function of the innate and adaptive immune systems still need to be explore, with regard to the risk of autoimmune diseases in general and CD and T1D in particular (9).

Study I

The rate of female and male CD patients in the Stockholm study cohort were similar: 38 girls and 39 boys with T1D had also been diagnosed with CD. This equal distribution between the sexes had been seen in other screening studies (175-177), but not all (118, 178).

In Study I, six of the eight children diagnosed with CD before T1D were girls. Unfortunately, there was not enough power to analyse these differences statistically.

A possible explanation for the overrepresentation of girls diagnosed with CD before T1D could be that girls showed more obvious symptoms, or that the symptoms and signs were recognized differently by the girl, her family and healthcare professionals.

Study II

Study II explored a population of Swedish children and adolescents diagnosed with T1D under the age of 17, who were born in 1992-1993 and 1997-1998, and this showed that 1,662/3,022 (55%) were boys. These results were consistent with previous studies that showed that males were slightly overrepresented in Swedish individuals with T1D (74). This tendency towards a more equal distribution, or a slight male overrepresentation, has been shown in other countries, which is interesting as females are overrepresented in the majority of other autoimmune diseases (9).

In general, no statistically significant differences by sex was found in the prevalence of CD in the T1D population. Overall, 173 males had received both diagnoses before the age of 17, compared to 164 females (CD prevalence 10.4% versus 12% and p-value 0.15). This finding in Study II agreed with Study I.

No differences according to sex were found in the epidemic cohort (52.9% male) compared to the post-epidemic cohort (49.1% male) (p-value 0.461). To our knowledge, no other studies are available that can provide comparable data about diagnoses of both CD and T1D during the epidemic, as we did not look at sex differences in the separately birth cohorts in Study I. Interestingly, the results from Study II did not agree with the cohort study regarding the general population in the Swedish CD epidemic (10), where the female to male ratio was more pronounced in the post-epidemic birth cohort, where 57 of the 89 children diagnosed with CD were girls (64%).

The results of Study II may indicate that the T1D population, regardless of the Swedish CD epidemic, were more at equally risk of developing CD, irrespective of the gender.

Study III

In Study III, the T1D population from the BBD study had an overrepresentation of boys (1,515/2,705, 56%), in agreement with previous mentioned review (9) and Study II.

The endpoint of Study III was the CD autoimmunity response at T1D diagnosis. The presence of the CD biomarker tTG at the time of T1D diagnosis, showed statistically significant differences in sex, with 84 girls (5.48%) showing CD autoimmunity, compared to 64 boys (4.31%) (p-value 0.0013). Sex differences were also seen when we analysed borderlines and positive values separately.

These findings indicated that CD autoimmunity was more frequent in girls at T1D onset. This sex difference needs to be explored further to evaluate if it maintains when CD diagnosis is set as the endpoint.

Study IV

Study IV showed male overrepresentation in the T1D cohort, as in previously mentioned review (9) and Studies II and III in this thesis.

With regard to CD autoimmunity in Study IV, we found that 77 females and 74 males had positive tTG, while the prevalence of CD autoimmunity was slightly higher at 8.4% for females and 6.6% for males, but it was not statistically significant (p-value 0.12).

The overall female to male ratio was similar regarding the children with both CD and T1D. The female to male ratio of children diagnosed with CD before T1D diagnosis was not statistically significantly different, as 17 out of the 32 children were females. Furthermore, among the 2,003 children with T1D screened for CD, 55 females and 58 males were diagnosed with CD. In addition, only one of the children with positive anti-tTG and normal biopsies was a girl. Thus, of the 145 children and adolescents with CD and T1D, 72 were girls and 73 were boys (p-value 0.2) (Table 7).

	Number of females (%)	Number of males (%)	Population female/male	P-value
Celiac disease before diabetes	17 (1.85)	15 (1.34)	915/1120	0.3
Celiac diagnosed via screening	55 (6.13)	58 (5.24)	897/1106	0.4
Total celiac disease prevalence	72 (7.86)	73 (6.52)	915/1120	0.2

***Table** 7. Prevalence according to sex. P-values > 0.05 were consider non-significant.*

This means that the results in study IV were consistent with the results in Studies I and II when it came to sex ratio.

Study IV had CD diagnosis as the endpoint and there was considerable overlapping between this study and study III with regard to the subject who took part in them. The differences in

the results can be attributed to different methodologies. Study III only considered CD autoimmunity at T1D diagnosis and included some children with known CD before the T1D diagnosis. In contrast, Study IV adopted a CD case-finding approach after the T1D diagnosis.

In summary, in contrast to other autoimmune diseases, boys with T1D were in majority compare to girls, and seemed to have the same risk to develop CD as girls with T1D. This risk was not influenced in either sex for individuals born during or after the Swedish CD epidemic.

6.6 REFLECTIONS ON AGE DATA

Study I

The mean age at diabetes diagnosis was 6.8 years (range 0.17 to 16.7 years) among the children with both CD and T1D. This included the children with CD diagnosed before T1D and the children diagnosed by screening or due to symptoms. The mean age at CD diagnosis was 8.3 years (range 0.5 to 19.4 years).

Study II

Overall, the children and adolescents with T1D in the post-epidemic cohort were diagnosed with CD at a statistically significant younger age (mean 9.4 years) compared with children and adolescents born during the epidemic (11.0 years) (p-value 0.002).

The age at CD diagnosis in T1D according to sex showed some statistically significant differences. In the post-epidemic cohort, the boys were 2.1 years younger at CD diagnosis, with a mean of 9.8 years compared to 11.9 years in the epidemic cohort (p-value 0,003). Also, the girls in the post-epidemic cohort were 1.1 year younger (9.1 years) compared to girls born during the epidemic (10.2 years). However, this was not statistically significant different (p-value 0.127).

While there was no statistically significant difference at age at diagnosis of T1D for the children with CD between the cohorts (8.3 years compared to 8.5 years, p-value 0.707), the mean age at T1D diagnosis was significantly lower in the group diagnosed with both T1D and CD than in children diagnosed with T1D only (8.4 years compared to 9.8 years, p-value <0.001).

Study III

We studied the presence of the CD biomarker tTG in this T1D population. The median age of the children at T1D diagnosis was 10.1 years, with a slightly sex difference variation: 9.9 years in girls and 10.9 in boys.

More than half of the T1D children were in the age range between 5-14. The presence of tTG in these different groups showed no significant difference (Figure 23).

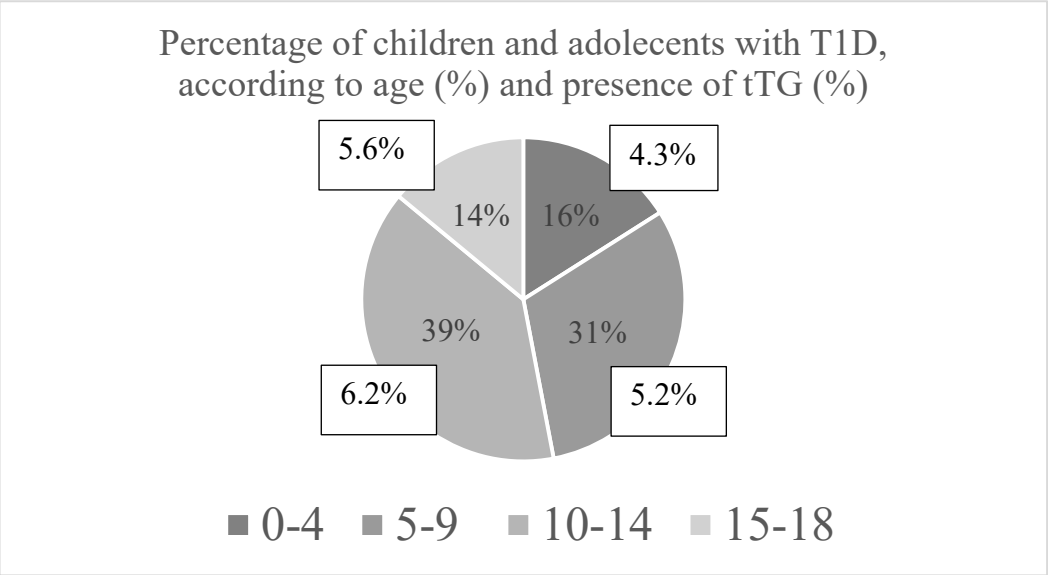

Figure 23. *Description of the proportions of age groups in the T1D population, and the percentage of the children and adolescents with tTG positive or borderline.*

We appreciated a tendency to a lower percentage in the youngest group. Subsequently, we controlled the subgroup of children younger than two years of age (n=76, 2.8% of the study population), and found that none of these young children had positive tTG, and only two had borderline tTG.

Study IV

With regard to the prevalence of CD by age range in children withT1D in Study IV, we calculated age, distribution and proportion of screened children. There was no normal distribution and the number of very young children was small (Figure 24 and Figure 25).

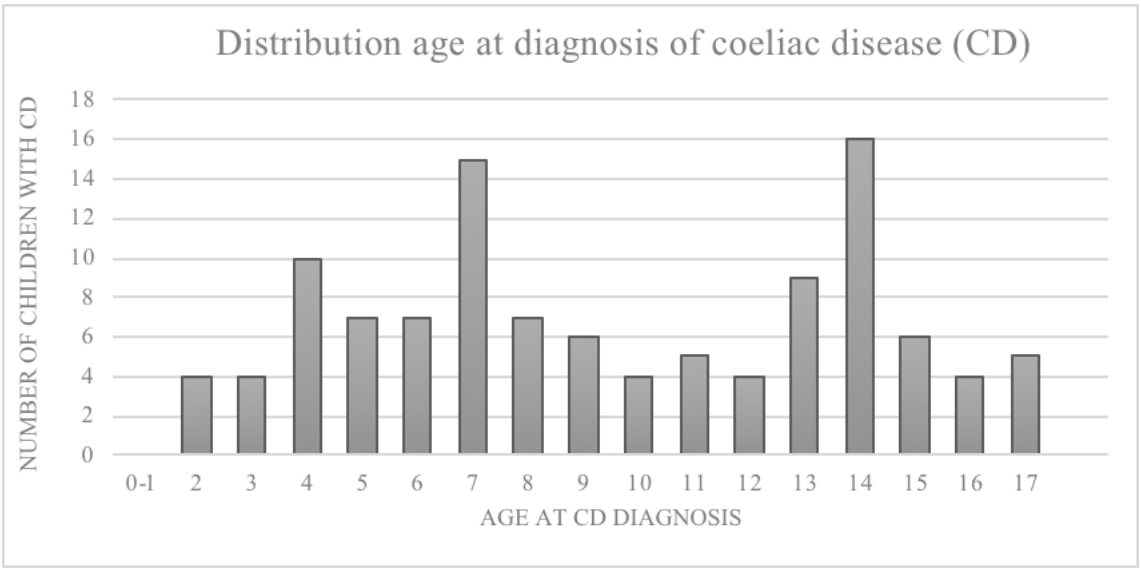

Figure 24. *Distribution of age at diagnosis of CD, by screening, in 113 children and adolescents with T1D.*

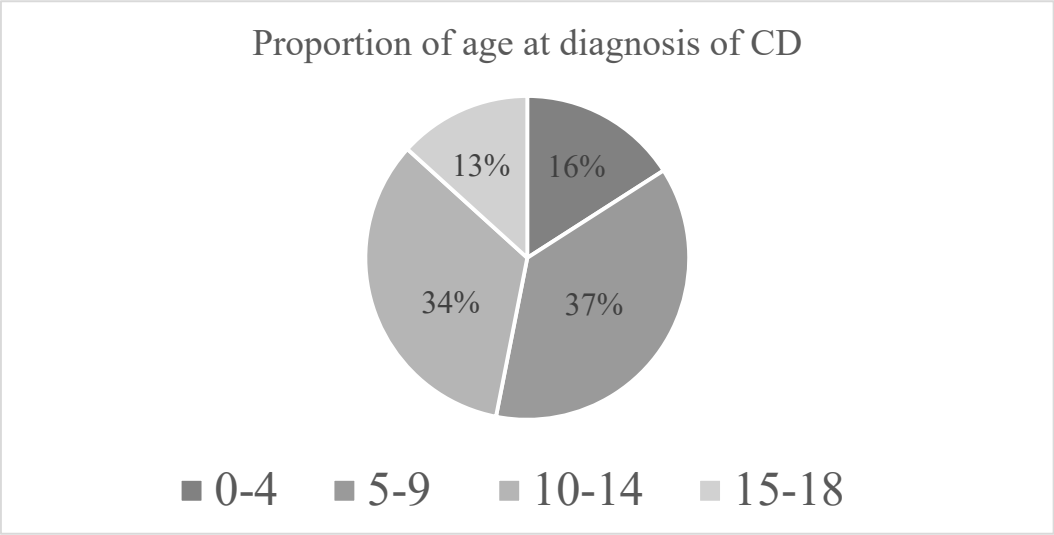

***Figure 25**. Proportion of age at diagnosis of CD, diagnosed by screening, in the 113 children and adolescents with T1D.*

When we compared Figure 24 with Figure 25, we noted that the proportion with a CD diagnosis by screening seemed to be following the total amount of children with T1D in each category.

As expected, we found a difference in mean age between the children diagnosed with CD before T1D and the children diagnosed with CD after the T1D diagnosis (Table 8).

***Table 8**. Age at diagnosis of T1D and CD, and interquartile range (IQR), with the low 1st quartile and the high 3rd quartile in brackets.*

	Age at T1D diagnosis and IQR	Age at CD diagnosis and IQR	Number of children
Children with CD before T1D	10.3 (7.4-13.1)	4.75 (1.6-6.2)	32
Children with T1D and CD diagnosed via screening	7.9 (4.2-12.9)	9.1 (6.2-14.1)	113

Age differences were evaluated in various aspects. One finding was that the individuals with T1D and CD were statistically significant younger at the T1D diagnosis than the individuals with T1D that did not developed CD in childhood. This observation was in accordance with some previous studies (118, 167, 178, 179), but not all (175, 180). This highlighted the need for more research in this aspect.

Furthermore, the children in the post-epidemic cohort were diagnosed with CD at a younger age. One possible explanation is that the 1997-1998 cohort may have been screened more often for CD at T1D diagnosis and for the first years after that than the epidemic cohort. The possible causes for this could be that screening in children with T1D was less common in the beginning of the 1990's, the referral sometimes was postponed, and the general awareness for CD in T1D had increased towards the end of the decade.

6.7 PROPOSAL FOR FUTURE SCREENING

The findings in this thesis supports that the screening procedure for diagnosing CD in children and adolescents with T1D can be improved.

From Study I we can suggest that all children with T1D should be screened, at the time of T1D diagnosis, and at least the first two years after that. In Studies III and IV we found that children without high-risk HLA may not need further screening. In addition, in Study IV, we showed that tTG levels that were 10 times above the ULN were a reliable way to diagnose CD and avoid invasive biopsies.

Figure 26 is a simplified proposal for a future screening procedure.

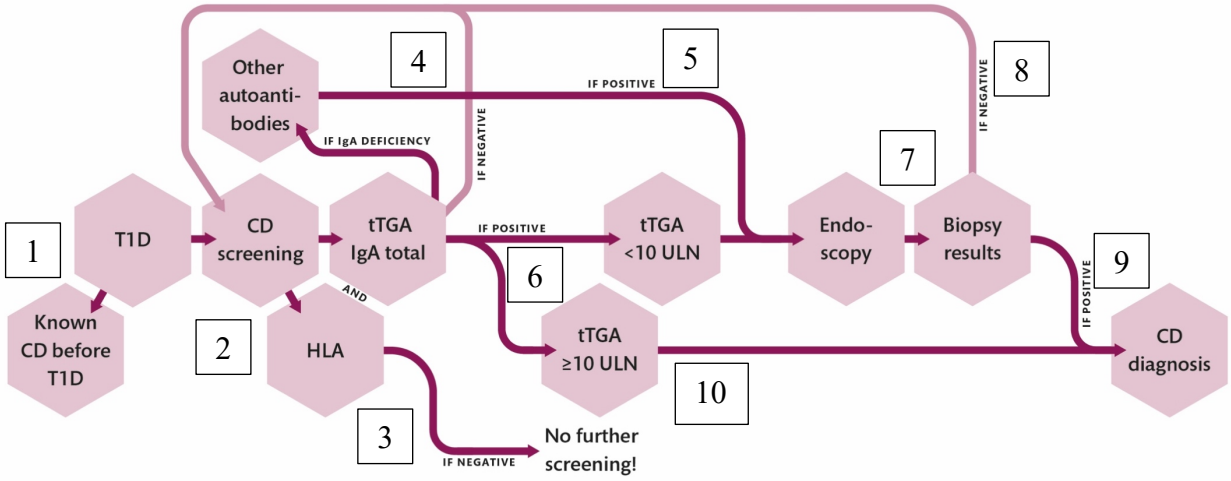

Figure 26. *A simple proposed algorithm for diagnosing CD by screening children and adolescents with T1D.*
1. Children and adolescents diagnosed with CD before their T1D diagnosis do not need to be screened. The others should be screened.
2. The CD screening includes CD biomarker tTG antibodies, total IgA and HLA genotyping.
3. If the children do not have HLA DQ2 or HLA DQ8 then further screening for CD is not necessary.
4. If the CD biomarker is negative, the screening should be repeated later, preferably during the first two years after their T1D diagnosis. If IgA deficiency is diagnosed, then other biomarkers of IgG type should be used for screening for CD.
5. If children have an IgA deficiency, and other CD biomarkers were positive, they should be referred for an endoscopy to retrieve biopsies.
6. Children with positive tTG autoantibodies should be divided into two groups depending of the level of the CD biomarker, namely under and over 10 times the limit of normal.
7. Children with tTG under 10 times the limit of normal should have an endoscopy to retrieve biopsies.
8. If the biopsy results are normal, the screening should be repeated later. How often, and after how long, has not been decided.
9. If the biopsy results show mucosal damage consistent with CD, the diagnosis should be given.
10. Children with tTG over 10 times above the limit of normal, in two separate samples, can receive a CD diagnosis without an invasive procedure with endoscopy.

7 CONCLUDING REMARKS

A high prevalence of CD in Swedish children and adolescents with T1D was found, both in Stockholm and at a national level. Every tenth child and adolescent with T1D in Sweden also had CD. The majority of children diagnosed with CD had positive CD biomarkers at the diagnosis of T1D or seroconverted during the first few years after that. We therefore recommend that patients are screen for CD when they are diagnosed with T1D, and, if that screening is normal, they should be tested at least yearly the first two years after that.

The prevalence of CD during and after the Swedish CD epidemic was found to be similar in children and adolescents with T1D. Thus, the T1D population at high-risk for developing CD may not be affected by environmental factors as the general population. This knowledge may be considered when planning both long-time observational studies regarding trigger factors for CD and interventional studies that aim to prevent CD.

HLA typing, which was included in the screening procedure for diagnosing CD, played a limited role in children and adolescents with T1D. Thus, HLA typing could be reserved for identifying the patients that would not be at-risk for developing CD and could avoid recurrently screening for CD. This would be approximately 8% of the T1D population.

High levels of the CD biomarkers tTG in the peripheral blood was prognostic for CD in children and adolescents with T1D. When the CD biomarker tTG was 10 times above the ULN it reliably prognosed CD. Approximately, half of the children and adolescents with T1D could thus avoid pre-anaesthesia fasting and invasive biopsies, and healthcare costs could be reduced. We suggest that children and adolescents with T1D should be included in national and international guidelines regarding CD diagnostic algorithms.

8 FUTURE PERSPECTIVES

The overall aim of the studies included in this thesis was to provide more knowledge about CD in children and adolescents with T1D, but there is still so much more that I want to know. I want to continue my research in order to understand more about what triggers CD and T1D and their co-existence.

CD screening has already been improved in clinical practice, due to the introduction of new guidelines. However, some important aspect of the optimal timing for CD biomarker tests remain unresolved. One main challenge is to validate how often, and for how long after their T1D diagnosis, children and adolescents should be screened for CD.

It may be possible to stratify, and adapt, screening schedules for CD in a more individualized way. As CD is more common in children diagnosed with T1D earlier in life, younger children may need to be screened more often, and for a longer period of time, than adolescents who are diagnosed with T1D. I am also interested in validating if the different risks posed by HLA genes, together with age of onset of T1D, could contribute to a more individualized screening algorithm.

Furthermore, several aspects of the accuracy of tTG as a predictor for CD need to be investigated. For example, it would be interesting to validate the no biopsy approach in children and adolescents with T1D, which is proposed in this thesis, in other non-Swedish T1D populations. Another aspect of our results that needs to be followed up is whether tTG that is seven times above the ULN would predict CD in other T1D and non-T1D populations. I would also like to explore how low tTG levels change over time after a T1D diagnosis and their predictive value for diagnosing CD in T1D populations. Multicentre and multinational collaborations, using only one type of serology test for tTG, would provide a good research approach.

Nevertheless, diagnosis with serology (CD biomarkers) and histology (biopsies) is not perfect. The diagnostic pathway depends on whether the patients are on a diet that contains gluten and, even then, the histopathological changes can be mild, or patchy, which poses specific diagnostic challenges. That is why new diagnostic tools and methods are more than welcome. For example, the immunological effects of pathogenic gluten peptides in peripheral blood after oral gluten challenges needs to be measured in children and adolescents with T1D. Another method that needs to be validated in the T1D population is using video capsule endoscopy to complement serological biomarkers when diagnosing CD. With regard to the future, nanotechnology could offer new solutions for both diagnostic accuracy and maybe even for immunomodulation and disease control.

The Swedish CD epidemic did not affect the prevalence of CD in children at-risk for diabetes in the otherwise healthy population. It would be interesting to find out if there were any

differences in the incidence of T1D during and after the Swedish CD epidemic. We hope that the result of our ongoing register study will be able to answer this question.

In parallel, studies to assess whether the prevalence of CD in other at-risk populations was affected by the Swedish CD epidemic in childhood will provide more information about the pathogenesis of CD. For example, a study using several Swedish registries could compare first-degree family members to individuals with CD.

There are a number of other important, unresolved research challenges. A key question that needs to be answered is how to prevent the development of CD, especially in high-risk groups. Several ongoing longitudinal prospective birth-cohorts' studies may prove helpful in establishing this in the near future.

Last, but not least, I would like to explore how to make daily life easier for children and adolescents with co-existing CD and T1D. Studies about how children and adolescents comply with their gluten-free diet, and the specific challenges they face, may improve their clinical management. In addition, new technology and telemedicine could provide additional support. We also need to evaluate overall, and disease-specific, quality of life tools for children and adolescents with both CD and T1D. This would enable us to compare them with children and adolescents with just CD or T1D. Exploring these important fields would enable us to shine some light on the complexity of living with both CD and T1D.

9 POPULAR SCIENCE RESUME

Coeliac disease in children and adolescents with type 1 diabetes – Screening, diagnosis and prevalence

Many children with type 1 diabetes have coeliac disease
Coeliac disease (CD) is more common in children and adolescents with type 1 diabetes (T1D). We explored the prevalence of CD in children and adolescents with T1D and found that one in ten children with T1D was also diagnosed with CD.

Coeliac disease
CD is a medical condition that leads to intestinal damage. It is the mucosa of the small intestine that becomes inflamed and that inflammation is due to the body´s reaction towards gluten. The body misunderstands the signals and causes damage to the intestinal mucosa, which is completely or partially destroyed. "Autoimmune disease" refers to the kind of illness where our immune system attacks and destroys healthy cells in our body. CD is therefore an autoimmune disease.

CD is a common condition in the general population. About one in 100 people in the Western world suffers from this disease. CD is more common among women and two out of three patients are female. This is also one of the most common chronic diseases among children and adolescents in Sweden.
The number of people diagnosed with CD depends on several different factors. The number of children with CD is different in different regions, both within Sweden and among neighbouring countries. The prevalence also varies within the same population. In Sweden, there was a dramatic increase in CD in young Swedish children between 1984 and 1996, when the number of cases of CD quadrupled. This period was named "The Swedish epidemic of coeliac disease".

For the development of CD, gluten intake is required, as well as a combination of genetic predisposition and environmental factors. Today, knowledge is still insufficient as to why some people with proven heredity develop the disease, whereas others do not.

The investigation of suspected CD can be started with a blood test. Nowadays, the mostly used antibodies are called anti-tissue transglutaminase (tTG). The test results determine how to proceed in order to get the diagnosis. The diagnostic protocol includes a medical examination with a flexible tube (gastroscope) that goes through the mouth into the small bowel. Through the gastroscope, small tissue samples (biopsies) are taken from the small bowel. The biopsies are then analysed under a microscope to assess intestinal damage. If tTG levels are found to be very high in two separate samples, the blood tests results are considered sufficient to diagnose CD.

The possibility of avoiding biopsies is a real advantage. On the one hand, it is beneficial for the child, who does not need to fast and be subjected to anaesthesia and a gastroscopy examination. It provides advantages for the family, because the diagnosis that is just based on blood samples is less time-consuming. In addition, it saves the healthcare system time and uses more cost-effective methods to provide a diagnosis, without the need to perform invasive procedures to take biopsy samples.

Type 1 diabetes
T1D is a chronic disease that makes the body unable to metabolize sugar, which is our most important source of energy. The hormone insulin works like a key that opens the door for sugar to enter the cell as fuel

T1D is an autoimmune disease. The ß-cells in the pancreas, where the insulin is produced, are damaged. When many of these cells stop working, the sugar stays in the blood without entering the cells, causing the blood sugar to become high. When the blood sugar level becomes steadily higher than normal, the condition is called diabetes.

The prevalence of T1D in Sweden is the second highest in the world. Only Finland has a higher figure. Around 50,000 individuals have T1D in Sweden and about 7,000 are children and young people. Every year, about 800 children develop the disease in Sweden.

For T1D to develop, a combination of genetic predisposition and environmental factors are required. Today, knowledge is still insufficient as to why some people with proven heredity develop the disease, whereas others do not.

T1D is diagnosed with blood tests—samples are also taken regarding diabetes autoantibodies, which can help to distinguish between different types of diabetes.

Coeliac disease in children and adolescents with type 1 diabetes
CD is more common in children and adolescents with T1D worldwide—the prevalence varies between 1.6% and 16%. In Sweden, other local studies showed that about 10% of children with T1D had CD.

The link between CD and T1D is explained by the fact that the diseases have a common heredity, as they share the same genes. The vast majority of children with T1D (approximately 90%) have risk genes for CD. The risk genes are human leukocyte antigen (HLA) DQ2 and DQ8. They are located on chromosome 6.

Due to the shared genetic risk between CD and T1D, children with T1D are screened for CD. A blood test to measure tTG levels is used for the screening and if the tTG levels are increased a biopsy procedure is the recommended next step. The reason for this recommendation is the lack of reliable studies showing it is also safe to diagnose CD in children with diabetes with repeated results of high tTG antibodies. To diagnose CD with just blood samples, as in children without T1D, would avoid pre-anaesthesia fasting for the children with T1D, and healthcare costs could be reduced.

Overall purpose of this dissertation and main objectives
The overall purpose of this dissertation was to expand current knowledge about CD in children and adolescents with T1D.
The specific objectives were as follows:

- To investigate the prevalence of CD in children and adolescents with T1D in Stockholm (Study I) and in Sweden (Study II).
- To compare the prevalence of CD in children with T1D born before (Study I), during and after (Studies I and II) the Swedish CD epidemic,
- To find out if CD screening in children and adolescents with T1D could be improved (Studies I, III and IV),
- To elucidate risk genes for CD in children and adolescents recently diagnosed with T1D, in relation to the coeliac biomarker tTG and diabetes- specific autoantibodies (Study III).
- To investigate if the no biopsy approach can be safe to diagnose CD in children and adolescents with T1D (Study IV).

Research strategy
In the first study, we examined the records for 1,151 paediatric patients at a diabetes clinic in Stockholm. We determined the number of children with T1D who also had CD in that city. We divided the patients into three subgroups: children born before, during and after the Swedish CD epidemic, and performed a retrospective review of their medical records.

In the second study, we wanted to expand the data and investigate this relation at a national level and confirm the results from the first study. Therefore, we investigated the diagnoses T1D and CD in several Swedish databases. All people in Sweden who receive care in a hospital receive one or more diagnoses. These diagnoses are collected as codes in national databases. In addition, all individuals with diabetes are offered to participate in a database concerning diabetes in particular. We created two groups: individuals born during the Swedish CD epidemic (1992–1993) and those born after the epidemic (1997–1998). The objective was to study those who developed T1D as a child and who also got the diagnosis of CD.

In the third study, we used parts of a Swedish prospective cohort study of children and adolescents with diabetes. The study is called Better Diabetes Diagnosis (BDD) and covers virtually all children and adolescents under the age of 18 who have been diagnosed with diabetes in Sweden since 2005. We examined blood samples from 2,705 children and adolescents with T1D when they were diagnosed with T1D. The blood samples were analysed to find links between the risk genes HLA DQ2 and DQ8, the coeliac biomarker tTG and the diabetes-specific autoantibodies IAA, GADA, IA2A and ZnT8.

In the fourth study, we also used BDD as a study base. We analysed information regarding 2,035 children and adolescents with T1D from the medical records kept by their diabetes clinics. In this way, we were able to describe the diagnostic procedures for the children who were also diagnosed with CD.

All studies were approved by ethical review committees in Sweden.

Results and implications

We confirmed a high prevalence of CD in Swedish children and adolescents with T1D, both in Stockholm and at the national level. Every tenth child and adolescent with T1D in Sweden also has CD. Many of the children who were diagnosed with CD had positive coeliac biomarkers already at the time they were diagnosed with T1D. In addition, the vast majority were diagnosed with CD during the first two years with T1D. We therefore recommend screening children with T1D for CD at diagnosis and at least for the first two years..

The prevalence of CD in children and adolescents with T1D was similar in children born during and after the Swedish CD epidemic. Thus, the population with T1D, who has a high-risk of developing CD, may not be affected by environmental factors, such as different amounts of gluten as the general population was . This knowledge can be considered when planning both long-term observational studies and interventional studies on the prevention of CD.

Genetic HLA tests for the risk genes DQ2 and DQ8 played a limited role in the diagnosis of CD in children and adolescents with T1D. Therefore, the determination of the HLA genes can be used to identify the approximately 8% of the T1D population who has no risk to develop CD. Consequently, these individuals do not need to undergo recurrent screenings.

High levels of the CD biomarker tTG predicted CD in children and adolescents with T1D. When the tTG was 10 times above the ULN it would have been safe and reliable to diagnose CD, without the need to confirm it with a biopsy. The children who met this requirement could thus avoid pre-anaesthesia fasting, and the gastroscopy procedure to take biopsies. Furthermore, a diagnosis without gastroscopy and biopsies is timesaving and reduces healthcare costs. Our suggestion is that national and international guidelines for the CD diagnosis should include the possibility to avoid biopsies in children with T1D.

10 POPULÄRVETENSKAPLIG SAMMANFATTNING

Celiaki hos barn och ungdomar med diabetes typ 1
Screening, diagnos och förekomst

Många barn med diabetes typ 1 har celiaki
Många barn som har diabetes typ 1 också har sjukdomen celiaki, som också kallas glutenintolerans. Vi har undersökt förekomsten av celiaki hos barn och ungdomar med diabetes typ 1 och påvisat att ett av tio barn i Sverige som har diabetes typ 1 har också celiaki.

Sjukdomen celiaki
Celiaki är en sjukdom med skador på tarmen. Det är slemhinnan i tunntarmen som blir inflammerad, och inflammationen beror på att kroppen reagerar mot gluten. Kroppen missförstår signalerna och det blir skador i tarmluddet, som helt eller delvis förstörs. Mekanismen som leder till sjukdomar där de egna cellerna förstör egna celler eller organ kallas för autoimmuna sjukdomar. Celiaki är en autoimmun sjukdom.

Celiaki är vanligt förekommande, cirka en person av hundra i västvärlden har glutenintolerans. Celiaki har ökat under de senaste trettio åren. Celiaki är vanligare i den kvinnliga delen av befolkningen –två av tre patienter är flickor eller kvinnor. Sjukdomen är dessutom en av de vanligaste kroniska sjukdomarna hos barn och ungdomar i Sverige. Hur många personer som får diagnosen celiaki beror på flera olika faktorer. Antalet barn med celiaki varierar i olika regioner, både inom Sverige, och mellan närliggande länder. Förekomsten av celiaki varierar också inom samma population. I Sverige förekom en dramatisk ökning av celiaki hos unga svenska barn mellan 1984 och 1996. Då fyrdubblades antalet fall. Denna tidsperiod kom att kallas ”Den svenska celiakiepidemin”.

För att celiaki skall utvecklas krävs intag av gluten, samt en kombination av arvsanlag och miljöfaktorer. Idag är kunskapen fortfarande otillräcklig angående varför vissa personer med påvisad ärftlighet får sjukdomen och andra inte.

Vid utredning av celiaki tar man blodprov. Nuförtiden används de antikroppar som kallas vävnadstransglutaminas (förkortas tTG). Vad som visas i provsvaret avgör hur man går vidare i diagnostiken. I det diagnostiska protokollet ingår en medicinsk undersökning där man via munnen för ned ett böjligt rör (gastroskop). Genom gastroskopet tas små vävnadsprover (biopsier) från tunntarmen och biopsierna analyseras sedan under mikroskop för att bedöma eventuell tarmskadan. Men om tTG visat sig vara mycket högt i två separata prov räcker dock blodprovsvaren för att kunna ge diagnosen.

Det är en fördel i diagnostiken att kunna avstå biopsier. Dels är det en vinst för barnet som inte behöver fasta och utsättas för narkos och en gastroskopiundersökning, och dels är det en vinst för familjen eftersom den medicinska undersökningen med enbart blodprover är mindre tidskrävande. Därutöver är det en vinst också för sjukvården då det är tidsbesparande och mer kostnadseffektivt om man kan undvara att behöva göra en biopsi.

Sjukdomen diabetes typ 1
Diabetes typ 1 är en autoimmun sjukdom som leder till att kroppen inte längre kan ta vara på socker, som är en viktig energikälla. Hormonet insulin är nyckeln som öppnar dörren för att sockret ska komma in i cellen så att kroppen får en normal ämnesomsättning. Om cellerna i bukspottskörteln, där insulinet tillverkas, skadas och slutar att fungera, stannar sockret kvar i blodet. Då blir blodsockernivån högre än normalt och det tillståndet kallas diabetes.

Förekomsten av diabetes typ 1 i Sverige är den nästa högsta i världen. Bara Finland har högre siffror. Runt 50000 individer har typ 1 diabetes i Sverige, och av dessa är ca 7000 barn och ungdomar. Per år insjuknar ca 800 barn i Sverige. Antalet barn och ungdomar med diabetes typ 1 har ökat över tiden. I början av 2000-talet såg man en fördubbling jämfört med 1980-talet. Ökningen har sedan dess avstannat något.

För att diabetes typ 1 skall utvecklas krävs en kombination av arvsanlag och miljöfaktorer. Idag är kunskapen fortfarande otillräcklig angående varför vissa personer med påvisad ärftlighet får sjukdomen.

Diabetes typ 1 diagnostiseras med ett blodprov. I samband med diabetesdiagnosen tas även prover angående diabetesautoantikroppar som kan hjälpa till att skilja mellan olika typer av diabetes.

Celiaki hos barn och ungdomar med diabetes typ 1

Sjukdomen celiaki är vanligare hos barn och ungdomar med diabetes typ 1. Antalet barn som har båda sjukdomarna varierar globalt mellan 1,6 % och 16 %. I Sverige har cirka 10 % av barn med diabetes typ 1 också celiaki.

Sambandet mellan celiaki och diabetes typ 1 förklaras med att sjukdomarna har gemensam ärftlighet, att de delar samma gener. De allra flesta barn med diabetes typ 1 (cirka 90 %), har riskgener för celiaki. Riskgenerna är: humant leukocytantigen (HLA) DQ2 och DQ8. De finns på ett område i arvsanlaget, på kromosom 6.

På grund av det genetiska sambandet mellan sjukdomarna undersöker vi idag barn med diabetes typ 1 för att se om de också har celiaki. Det kallas att screena. Även här tar man blodprov för att mäta antikropparna tTG, men även om tTG är mycket högt i två prover i rad är det dock inte rekommenderat att redan där ge diagnosen celiaki. Man går istället vidare med biopsier. Anledningen till det är att man saknat undersökningar som visar att det är tillförlitligt att ge diagnosen celiaki när barn med diabetes typ 1 har höga tTG. Med en undersökning utan biopsier skulle vissa barn kunna undvika att fasta före anestesin och själva endoskopiundersökningen, och det skulle vara mer kostnadseffektivt.

Övergripande syfte med denna avhandling och huvudmålen

Det övergripande syftet med denna avhandling var att utöka aktuell kunskap om celiaki hos barn och ungdomar med diabetes typ 1.

De specifika målen var följande:

- Att undersöka förekomsten av celiaki hos barn och ungdomar med diabetes typ 1 i Stockholm (studie I) och i Sverige (studie II).
- Att jämföra förekomsten av celiaki hos barn med diabetes typ 1 födda under den svenska celiakiepidemin, dels med barn med diabetes typ 1 födda före nämnda epidemi (studie I), och även med barn födda efter (studier I och II).
- Att ta reda på om celiaki-screeningen hos barn och ungdomar med diabetes typ 1 skulle kunna förbättras (studier I, III och IV), och i så fall hur.
- Att undersöka riskgenerna HLA DQ2 och DQ8 hos barn och ungdomar som nyligen diagnostiserats med diabetes typ 1, samt att belysa dessa geners förekomst i relation till celiaki-biomarkören tTG och autoantikroppar vid diabetes (studie III).
- Att utreda om det kan vara säkert att diagnostisera celiaki hos barn och ungdomar med diabetes typ 1 utifrån endast blodprover, utan en medicinsk undersökning med gastroskopi och biopsier (studie IV).

Forskningsstrategi

I den första studien undersökte vi journalerna för 1151 barn på en diabetesklinik i Stockholm. Vi tog reda på hur många av barnen med diabetes typ 1 som hade celiaki. De av barnen som hade båda sjukdomarna delade vi in i tre undergrupper: barn födda före, under och efter den svenska celiakiepidemin, och utförde sedan en omfattande retrospektiv granskning av deras journaler.

I den andra studien ville vi utöka underlaget och ta reda på hur det låg till på ett nationellt plan. Vi ville bekräfta resultaten från första studien avseende både det totala antalet barn och andelen av barn och ungdomar med celiaki bland barnen med diabetes typ 1, både under och efter den svenska epidemin. Därför gjorde vi en analys av existerande diagnoser i olika svenska databaser. (Alla personer i Sverige som får vård på sjukhus får en eller flera diagnoser. Dessa diagnoser samlas som koder i nationella databaser. Dessutom erbjuds alla individer med diabetes att delta i en databas angående just diabetes). Vi skapade två grupper: individer födda under den svenska celiakiepidemin, 1992–1993, och individer födda efter densamma, 1997–1998. Detta för att studera vilka som fått diabetes typ 1 som barn, och bland dem, vilka som också fick celiakidiagnosen.

I den tredje studien använde vi delar av en svensk prospektiv studie av barn och ungdomar med diabetes. Studien heter Bättre Diabetes Diagnos (BDD), och omfattar i stort sett alla barn och ungdomar under 18 år

som fått diagnosen diabetes i Sverige sedan 2005. Vi undersökte blodprover från 2705 barn och ungdomar med diabetes typ 1, vilka tagits i samband med diabetesdiagnosen. Blodproverna analyserades avseende kopplingarna mellan riskgenerna HLA DQ2 och DQ8, celiaki-antikroppar tTG och autoantikroppar för diabetes (IAA, GADA, IA2A och ZnT8).

Även i den fjärde studien använde vi BDD som studiebas. Vi kombinerade information angående 2035 barn och ungdomar med diabetes typ 1 med journaldata från barnens respektive diabetesklinik. På så vis kunde vi analysera vilka, hur och på vilket sätt barnen fått sin celiakidiagnos. Vi undersökte om det kan vara tillförlitligt att diagnostisera celiaki hos barn och ungdomar med diabetes typ 1 utifrån endast blodprover.

Alla studier godkändes av etiska kommitteen i Sverige.

Resultat och implikationer

Vi bekräftade en hög förekomst av celiaki hos svenska barn och ungdomar med diabetes typ 1, både i Stockholm och på nationell nivå. Var tionde barn och ungdom med diabetes typ 1 i Sverige har även celiaki. Många av barnen som fick diagnosen celiaki hade positiva celiaki-biomarkörer redan i samband med att de fick sin diabetes typ 1-diagnos. Dessutom fick de allra flesta celiakidiagnosen under de första två åren med typ 1 diabetes. Därför rekommenderar vi att man screenar barn med typ 1 diabetes vid diabetesdiagnosen och åtminstone de första två åren efter diabetesdiagnosen.

Förekomsten av celiaki hos barn och ungdomar med diabetes typ 1 var densamma under och efter den svenska celiakiepidemin. Detta tyder på att populationen med diabetes typ 1 med hög risk för att utveckla celiaki, kanske inte påverkas av olika glutenmängder som den allmänna befolkningen gör. Denna kunskap kan tas med i beräkningen när man planerar både långvariga observationsstudier och interventionella studier avseende förebyggandet av sjukdomen celiaki.

HLA riskgenerna DQ2 och DQ8 hade en begränsad roll i samband med diagnosen av celiaki hos barn och ungdomar med diabetes typ 1. Därför kan bestämningen av HLA generna användas för att identifiera de cirka 8 % av diabetes typ 1-populationen som inte är i riskzonen för att utveckla celiaki. Dessa individer behöver följaktligen inte genomgå återkommande celiaki-screeningar.

Höga nivåer av celiaki-biomarkören tTG prognosticerade celiaki även hos barn och ungdomar med diabetes typ 1. När celiaki-biomarkörerna tTG var över tio gånger den normala gränsen var det tillförlitligt att diagnostisera celiaki, utan att bekräfta det med en biopsi. Barnen som uppfyllde detta krav med höga celiaki-biomarkören tTG skulle därför kunna undvika gastroskopin för att ta biopsier. Celiakidiagnos utan biopsi är skonsamt för barnet och familjen. Vidare är en diagnos utan gastroskopi och biopsier tidsbesparande och minskar hälso- och sjukvårdskostnaderna. Vi rekommenderar att riktlinjer för celiakiutredningen hos screenade barn skulle också kunna gälla för barn med diabetes typ 1, angående kriterierna när biopsier kan undvaras.

11 RESUMEN CIENTÍFICO DIVULGATIVO

Enfermedad celíaca en niños y adolescentes con diabetes tipo 1
Cribado, diagnóstico y prevalencia

Numerosos niños con diabetes tipo 1 padecen también la enfermedad celíaca
La enfermedad celíaca (EC) es más habitual en niños y adolescentes con diabetes de tipo 1 (T1D). Hemos estudiado la prevalencia de la EC en niños y adolescentes con T1D y hemos observado que uno de cada diez menores con diabetes de tipo 1 también ha sido diagnosticado de enfermedad celíaca.

Enfermedad celíaca
La EC es un trastorno que provoca daños intestinales. La mucosa del intestino delgado se inflama debido a la reacción del organismo hacia el gluten. El organismo no interpreta correctamente las señales y provoca daños en la mucosa intestinal, la cual se destruye de forma total o parcial. Se entiende por «enfermedad autoinmune» un tipo de enfermedad en la que nuestro sistema inmunológico ataca y destruye las células sanas de nuestro cuerpo. La EC es, por lo tanto, una enfermedad autoinmune.

La EC es un trastorno común en la población; en torno a una de cada cien personas en el mundo occidental la sufre. Es más habitual entre las mujeres: dos de cada tres pacientes lo padecen. Asimismo, se trata de una de las enfermedades crónicas más extendidas entre los niños y adolescentes de Suecia.

El número de personas a las que se les diagnostica la enfermedad celíaca depende de diversos factores. Por ejemplo, la cifra de menores con celiaquía es diferente en las distintas regiones, tanto en Suecia como entre los países vecinos. La prevalencia también varía dentro de la misma población. Por ejemplo, en Suecia se produjo un enorme aumento de la EC en la población infantil entre 1984 y 1996, ya que se cuadruplicó el número de casos de celiaquía. Se denomina a este periodo «la epidemia sueca de enfermedad celíaca».

Para que se desarrolle la EC se requiere la ingesta de gluten, así como una combinación de predisposición genética y factores ambientales. Hoy en día, todavía no se sabe a ciencia cierta por qué desarrollan la EC algunas personas cuya predisposición genética se ha constatado, mientras que otras no lo hacen.

La investigación de la sospecha de la EC puede iniciarse con un análisis de sangre. Hoy en día, los anticuerpos más utilizados se denominan antitransglutaminasa tisular (tTG). Los resultados de la prueba determinan cómo proceder para obtener el diagnóstico. El protocolo de diagnóstico incluye una prueba médica que se realiza con un tubo flexible (gastroscopio), el cual permite observar el intestino delgado a través de la boca. A través del gastroscopio, se toman pequeñas muestras de tejido (biopsias) del intestino delgado. Dichas biopsias se analizan a continuación con un microscopio para evaluar el daño intestinal. Si se detectan niveles de tTG muy elevados en dos muestras distintas, los resultados de los análisis de sangre se consideran suficientes para diagnosticar la enfermedad celíaca.

La posibilidad de evitar las biopsias constituye toda una ventaja. Por un lado, resulta beneficiosa para el niño, que no necesita ayunar y someterse no solo a la anestesia, sino también a una gastroscopia. Por otro lado, supone un alivio para la familia, ya que el diagnóstico verificado únicamente mediante muestras de sangre es más breve. Además, el sistema de salud puede ahorrar tiempo, al utilizar métodos más económicos que permiten ofrecer un diagnóstico sin necesidad de realizar un procedimiento invasivo para tomar muestras de biopsia.

Diabetes de tipo 1
La diabetes de tipo 1 es una enfermedad crónica que hace que el organismo ya no pueda metabolizar el azúcar, nuestra fuente de energía más importante. La hormona insulina funciona como una llave que abre la puerta para que el azúcar entre en las células a modo de combustible.

La diabetes de tipo 1 es una enfermedad autoinmune. Las células ß del páncreas, en las que se produce la propia insulina del cuerpo, están dañadas. Cuando muchas de estas células que producen insulina dejan de

funcionar, el azúcar permanece en la sangre sin penetrar en las células, lo que provoca que el nivel de azúcar en sangre se incremente. Cuando esto ocurre, el trastorno se denomina diabetes.

La prevalencia de la diabetes de tipo 1 en Suecia es la segunda más alta del mundo. Solo Finlandia tiene cifras más altas. Alrededor de 50 000 personas padecen T1D 1 en Suecia, de las que en torno a 7000 son niños y jóvenes. Cada año, unos 800 menores desarrollan la enfermedad en Suecia.

Para que se desarrolle la diabetes de tipo 1, se requiere una combinación de predisposición genética y factores ambientales. Hoy en día, todavía no se sabe a ciencia cierta por qué algunas personas con factores hereditarios comprobados desarrollan la diabetes de tipo 1, mientras que otras no lo hacen.

La diabetes de tipo 1 se diagnostica mediante análisis de sangre; también se toman muestras de los autoanticuerpos de la diabetes, que pueden ayudar a distinguir entre los diferentes tipos de diabetes.

Enfermedad celíaca en niños y adolescentes con diabetes de tipo 1

La celiaquía es más habitual en niños y adolescentes con diabetes de tipo 1 en todo el mundo: la prevalencia oscila entre el 1,6 % y el 16 %; en Suecia, ciertos estudios locales contemplan que en torno al 10 % de los menores con diabetes de tipo 1 padecen también la enfermedad celíaca.

El vínculo entre la EC y la T1D se explica por el hecho de que las enfermedades tienen un componente hereditario común, ya que comparten los mismos genes. La inmensa mayoría de los menores con diabetes de tipo 1 (aproximadamente el 90 %) poseen genes que les predisponen a desarrollar la enfermedad celíaca. Los genes de riesgo son el antígeno leucocitario humano (HLA) DQ2 y DQ8, que se encuentran en un área del genoma, en el cromosoma 6.

Debido al riesgo genético compartido entre la enfermedad celíaca y la diabetes de tipo 1, se realiza un cribado a los niños con diabetes de tipo 1 para conocer si son celíacos. La prueba se basa en un análisis de sangre que mide los niveles de tTG. Sin embargo, si los niveles de tTG son muy altos en dos pruebas distintas, no se recomienda que los niños y adolescentes con T1D obtengan el diagnóstico de enfermedad celíaca solo en función de dichas pruebas, a contrario de la recomendación para otros niños. La biopsia es el siguiente paso recomendado, y el motivo de esta recomendación es la falta de estudios fiables hasta este momento que demuestren que también es seguro diagnosticar la enfermedad celíaca en menores con T1D con niveles altos de anticuerpos tTG. Si se pudieses demostrar que se puede basar el diagnóstico únicamente en muestras de sangre, sin un examen médico que incluyera gastroscopia y biopsias, se podría evitar el ayuno previo a la anestesia a los niños con diabetes de tipo 1, al tiempo que se podrían reducir los costes sanitarios.

Propósito general de la presente disertación y objetivos principales

El propósito general de la presente disertación fue el de ampliar los conocimientos actuales sobre en niños y adolescentes con diabetes de tipo 1.

Los objetivos específicos fueron los siguientes:

- Investigar la prevalencia de la enfermedad celíaca en niños y adolescentes con diabetes de tipo 1 en Estocolmo (estudio I) y en Suecia (estudio II).
- Comparar la prevalencia de la enfermedad celíaca en niños con diabetes de tipo 1 nacidos durante la epidemia sueca de enfermedad celíaca, en parte con menores con diabetes de tipo 1 nacidos antes de la mencionada epidemia (estudio I), y también con niños nacidos después (estudios I y II).
- Conocer si se podría mejorar el cribado para la detección de celiaquía en niños y adolescentes con diabetes de tipo 1 (estudios I, III y IV) y, en caso afirmativo, investigar de qué manera.
- Investigar sobre los genes que predisponen a la enfermedad celíaca en niños y adolescentes a los que se ha diagnosticado recientemente diabetes de tipo 1, y dilucidar la presencia de estos genes en relación con el biomarcador celíaco tTG y la presencia de autoanticuerpos en la diabetes (estudio III).
- Investigar si el enfoque sin biopsia podría ser seguro para diagnosticar la enfermedad celíaca en niños y adolescentes con diabetes de tipo 1 (estudio IV).

Estrategia de investigación

En el primer estudio, examinamos los historiales de 1151 pacientes pediátricos en una clínica de Estocolmo especializada en diabetes. Llegamos a la conclusión de que numerosos niños con diabetes de tipo 1 padecen también la enfermedad celíaca en dicha ciudad. Asimismo, dividimos a los pacientes con ambas enfermedades

en tres subgrupos, compuestos por niños nacidos antes, durante y después de la epidemia sueca de celiaquía, respectivamente. A continuación, realizamos una revisión retrospectiva exhaustiva de sus historiales médicos.

En el segundo estudio, quisimos ampliar los datos e investigar esta relación a nivel nacional. Nuestro objetivo era confirmar los resultados del primer estudio sobre el número total de niños, así como sobre la proporción de niños y adolescentes con enfermedad celíaca entre los menores con diabetes de tipo 1, durante y después de la epidemia sueca. Así, realizamos un análisis de los diagnósticos de EC y T1D existentes en varias bases de datos suecas. (Todas las personas que reciben atención en un hospital de Suecia reciben uno o más diagnósticos; tales diagnósticos se recogen en forma de códigos en las bases de datos nacionales. Además, se ofrece a todos los individuos con diabetes la posibilidad de aparecer en una base de datos relativa a la diabetes en particular). Creamos dos grupos: los individuos nacidos durante la epidemia sueca de celiaquía (1992-1993) y los nacidos después de la epidemia (1997-1998). El objetivo era estudiar quiénes desarrollaron diabetes de tipo 1 durante la infancia y, de ellos, quiénes recibieron también el diagnóstico de la enfermedad celíaca.

En el tercer estudio, usamos partes de un estudio sueco prospectivo de cohortes que se llevó a cabo con niños y adolescentes diabéticos. El estudio se denomina Better Diabetes Diagnosis (BDD) y abarca prácticamente a todos los niños y adolescentes menores de 18 años diagnosticados de diabetes en Suecia desde 2005. Examinamos muestras de sangre de 2705 niños y adolescentes con diabetes de tipo 1 cuando recibieron el diagnóstico de esta enfermedad. Se analizaron las muestras de sangre para encontrar vínculos entre los genes de riesgo HLA DQ2 y DQ8, el biomarcador de celiaquía tTG y los autoanticuerpos específicos en la diabetes (IAA, GADA, IA2A y ZnT8).

Para el cuarto estudio también se recurrió al estudio BDD: combinamos los datos relativos a 2035 niños y adolescentes con diabetes de tipo 1 con los datos de los historiales médicos de las clínicas especializadas en diabetes. De esta manera, pudimos analizar qué niños fueron diagnosticados de enfermedad celíaca, así como describir la vía y el modo para llegar a dicho diagnóstico.

Todos los estudios recibieron la aprobación de comités de revisión ética de Suecia.

Resultados e implicaciones

Se confirmó una alta prevalencia de la enfermedad celíaca en niños y adolescentes suecos con diabetes de tipo 1, tanto en Estocolmo como a nivel nacional. Uno de cada diez niños y adolescentes con diabetes de tipo 1 en Suecia también es celíaco. Muchos de los menores diagnosticados de enfermedad celíaca ya tenían biomarcadores de celiaquía positivos cuando se les diagnosticó la diabetes de tipo 1. Además, a la gran mayoría se les diagnosticó la enfermedad celíaca durante los dos primeros años con diabetes de tipo 1. Por lo tanto, recomendamos realizar el cribado de EC a los niños con diabetes de tipo 1 en el momento del diagnóstico y al menos durante los dos primeros años a partir de dicho diagnóstico.

La prevalencia de la enfermedad celíaca en niños y adolescentes con diabetes de tipo 1 fue similar en los niños nacidos durante y después de la epidemia sueca de enfermedad celíaca. Por tanto, la población con T1D, cuyo riesgo de desarrollar la EC es muy elevado, puede no verse afectada por factores ambientales, como las diferentes cantidades de gluten, como sí le sucedió a la población en general. Estos conocimientos pueden tenerse en cuenta al planificar tanto los estudios de observación a largo plazo como los estudios de intervención sobre la prevención de la enfermedad celíaca.

Las pruebas genéticas de HLA para los genes de riesgo DQ2 y DQ8 desempeñaron un papel limitado en el diagnóstico de la enfermedad celíaca en niños y adolescentes con diabetes de tipo 1. Por lo tanto, la determinación de los genes HLA se puede utilizar para identificar en torno al 8 % de la población diabética de tipo 1 sin predisposición a desarrollar la enfermedad celíaca. En consecuencia, estas personas no necesitan someterse a exámenes periódicos de cribado de enfermedad celíaca.

Los altos niveles del biomarcador celíaco tTG predijeron la enfermedad celíaca en niños y adolescentes con diabetes de tipo 1. Los biomarcadores de celiaquía tTG con niveles diez veces superiores al límite máximo de lo considerado normal resultaron fiables y seguros para diagnosticar la enfermedad celíaca de modo que la confirmación mediante biopsia no hubiese sido necesaria. Los niños que cumpliesen este requisito podrían así evitar el ayuno previo a la anestesia y el procedimiento de gastroscopia para tomar biopsias. Además, un diagnóstico sin gastroscopia y biopsias ahorra tiempo y reduce los costes de asistencia médica. En suma, sugerimos que las directrices nacionales e internacionales para el diagnóstico de la enfermedad celíaca incluyan la posibilidad de evitar las biopsias en menores con diabetes de tipo 1 en los casos aquí descriptos.

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