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Searching for Celiac Disease Screening-detected celiac disease in an HLA-genotyped birth cohort

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Screening-detected celiac disease in an HLA-genotyped birth cohort

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Title and subtitle

Searching for Celiac Disease. Screening-detected celiac disease in an HLA-genotyped birth cohort

Abstract

Objectives: Celiac disease is a common immune mediated enteropathy strongly associated with HLA-DQB1*02 (DQ2), *0302 (DQ8), or both and the presence of tissue transglutaminase autoantibodies (tTGA). Prevalence studies have revealed that most affected individuals go undetected because of subclinical signs or being asymptomatic rendering screening a method for identification. However, less is known about subclinical manifestations of screening-detected celiac disease during childhood and if these motivate identification and treatment. The overall aim of the present research was to identify children with screening-detected celiac disease in an HLA-genotyped birth cohort and to study systemic cytokines and bone mineral density (BMD) in these children.

Methods: Children were HLA-genotyped at birth and offered screening at three and nine years of age by detection of tTGA in plasma using radioligand binding assays. Children repeatedly positive for tTGA underwent intestinal biopsy to confirm diagnosis of celiac disease. The cytokines IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, and TNF-α were analysed at time of diagnosis and after treatment with a gluten-free diet and compared with matched controls. At nine years of age, children with screening-detected celiac disease were examined by dual X-ray absorptiometry for analysis of BMD and for serum 25(OH) vitamin D3 and plasma parathyroid hormone (PTH) and compared to matched controls.

Results: Screening-detected celiac disease was found in 3.5% (56/1618) of three year old children having HLA-risk alleles compared with none (0/1815) among children not having these risk alleles (p<0.001) (Paper I). A follow-up screening at nine years of age identified an additional 3.8% (72/1907) with celiac disease in the HLA-risk group compared with none (0/2167) in the control group (p<0.001) (Paper II). Three-year old children with screening-detected celiac disease had systemically elevated pro-inflammatory cytokines of both T_H1 and T_H2 pattern compared to controls of which most were down-regulated after starting a gluten-free diet (Paper III). At nine years of age, children with screening-detected celiac disease had lower BMD, lower levels of vitamin D but higher PTH levels compared with matched controls. In contrast, children on a gluten-free diet did not differ from their matched controls (Paper IV).

Conclusions: Screening-detected celiac disease is only found among children at genetic risk but repeated testing during childhood is necessary to detect new patients. HLA-genotyping could therefore be used to select large populations to be screened for celiac disease. Children with screening-detected celiac disease have systemically elevated pro-inflammatory cytokines and low BMD but normal values on a gluten-free diet, indicating that children with screening-detected celiac disease could benefit from early identification and treatment.

Key words Bone mineral density, Celiac disease, Children, Cytokines, DQ2, DQ8, DXA, HLA, Screening, tissue transglutaminase antibody

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Searching for Celiac Disease

Screening-detected celiac disease in an HLA-genotyped birth cohort

Sara Björck

To all children having celiac disease;
known and unknown.

Table of Contents

Abbreviations

AGA   anti-gliadin antibody

APC   antigen presenting cell

BMC   bone mineral content

BMD   bone mineral density

BMI   body mass index

CI   confidence interval

CiPiS   Celiac Disease Prediction in Skåne

DGP   deamidated gliadin peptide

DiPiS   Diabetes Prediction in Skåne

DXA   dual X-ray absorptiometry

ELISA   enzyme linked immunosorbent assay

EMA   endomysial autoantibodies

ESPGHAN   European Society for Paediatric Gastroenterology, Hepatology and Nutrition

HLA   human leukocyte antigen

HRQoL   health-related quality of life

IEL   intraepithelial lymphocytes

IFN   interferon

Ig   immunoglobulin

IL   interleukin

IR   incidence rate

MHC   major histocompatibility complex

PBM   peak bone mass

PCR	polymerase chain reaction
PTH	parathyroid hormone
RBA	radioligand binding assay
SNP	single nucleotide polymorphism
T1D	type 1 diabetes
T_H1	T helper cell type 1
TNF	tumour necrosis factor
tTG	tissue transglutaminase type 2
tTGA	tissue transglutaminase autoantibody
WHO	World Health Organisation

List of papers

This thesis is based on the following papers, which are referred to in the text as Paper I-IV:

I. **Sara Björck**, Charlotte Brundin, Ester Lörinc, Kristian Lynch, Daniel Agardh. Screening detects a high proportion of celiac disease in young HLA–genotyped children. *J Pediatr Gastroenterol Nutr. 2010 Jan; 50(1):49–53.*

II. **Sara Björck**, Kristian Lynch, Charlotte Brundin, Daniel Agardh. Repeated screening is necessary to detected celiac disease but can be restricted to at genetic risk birth cohorts. *J Pediatr Gastroenterol Nutr. Accepted for publication, August 2015.*

III. **Sara Björck**, Sabina Resic Lindehammer, Malin Fex, Daniel Agardh. Serum cytokine pattern in young children with screening detected coeliac disease. *Clin Exp Immunol. 2015 Feb; 179(2):230-235.*

IV. **Sara Björck**, Charlotte Brundin, Magnus Karlsson, Daniel Agardh. Reduced bone mineral density in children with screening-detected celiac disease: a case-control study. *In manuscript.*

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Background

Introduction

Early in my career as a medical student, and later on as a pediatrician, I learnt that celiac disease is a common immune-mediated enteropathy induced by dietary gluten ingestion in genetically predisposed individuals occurring throughout life. The classical symptoms of celiac disease in children are diarrhoea, abdominal distension and failure to thrive. A gluten-free diet, defined as a diet free from wheat, rye and barley, results in a recovery of the intestinal mucosa in the majority of affected individuals, but reintroduction of gluten into the diet results in relapse of the disease even after years of treatment.

During my first summer as a resident physician at the Children´s Hospital in Malmö, Sweden, I met a 3-year old girl approximately 3 years of age with chronic diarrhoea and tiredness. She was pale, had thin arms and legs and a large belly. I especially remember that her mother had to help her to get up from the examination table because she was weak and her belly prevented her from getting up. This was the first patient with classical appearance of celiac disease that I investigated - and the last. Since then, I have treated hundreds of children with celiac disease with a myriad of symptoms, but also children that are completely free from symptoms. This thesis is my contribution to the knowledge about celiac disease in children.

Pathogenesis

To date there are two known prerequisites for the development of celiac disease: having a genetic susceptibility for the disease and being exposed to gluten [1]. Since only a minority of genetically predisposed individuals that ingest gluten develop celiac disease, there are most likely environmental factors involved that may trigger the disease leading to the immune response to gluten [2] (**Figure 1**).
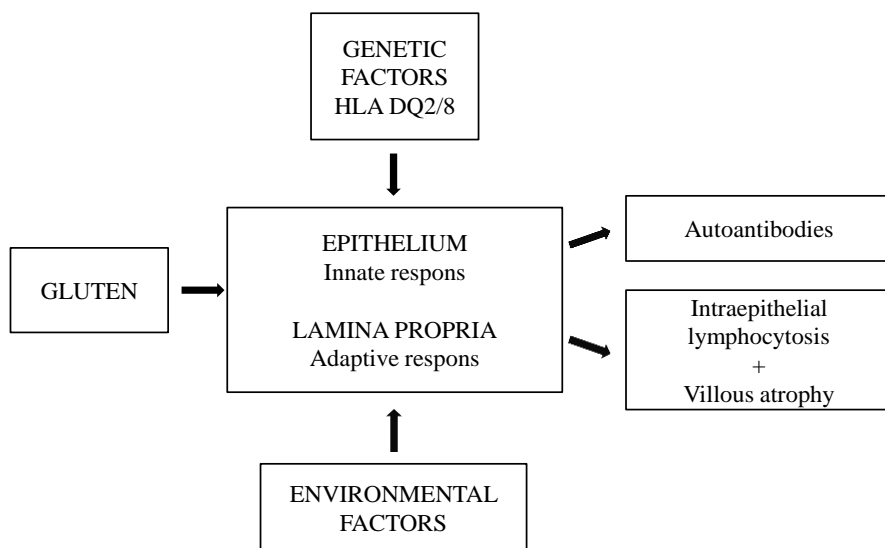
Figure 1. Schematic view of the major factors involved in the pathogenesis of celiac disease. The genetic susceptibility consists mostly of the HLA-DQ2 or DQ8 haplotypes and dietary gluten is the major exposure. In addition, there has to be environmental exposures which in combination elicit the typical immune response in the intestinal mucosa leading to production of autoantibodies such as tissue transglutaminase autoantibodies and the histological characteristics comprising of intraepithelial lymphocytosis and villous atrophy. Recreated from Green et al. J Allergy Clin Immunol, 2015. 135(5): 1099-1106 and published with permission from the publisher.

In celiac disease, two main antigens have been identified; gluten, the exogenous antigen of which is necessary for celiac disease to develop, respective tissue transglutaminase type 2 (tTG), the main autoantigen identified in 1997 to which autoantibodies are produced [3]. In contrast to other autoimmune diseases, the disease process can be reversed by eliminating gluten from the diet resulting in a reduction of tTG autoantibodies (tTGA) [4, 5].

Gluten

Wheat protein is divided into 4 categories based on their solubility in different solvents: albumins (soluble in water), globulins (soluble in dilute salt solutions), gliadins (soluble in aqueous alcohol) and glutenins (soluble in dilute alkali or acid) [6]. Gluten is often used as an aggregate name for the prolamin storage proteins of wheat (gliadins and glutenins), rye (hordeins) and barley (secalines).These proteins are after ingestion first degraded into large fragments by pepsin in the stomach, but due to the richness in the amino acids proline and glutamine the proteins are resistible to degradation by intestinal intraluminal and brush border endopeptidases and are only partially degraded into gliadin peptides before reaching the mucosa of the small intestine [7]. The spacing between proline and glutamine also plays a significant role in the deamidation by tTG [8]. On the

contrary, the prolamine proteins in oats, called avenins, contain much less amount of proline, which could explain why the majority of celiac disease individuals tolerate pure oats [9].

Genetics

The observation that celiac disease is more common in relatives to celiac disease individuals and the high concordance rate between monozygotic twins indicate a strong genetic influence on disease risk [10]. The first observed genetic association was to certain alleles in the human leukocyte antigen (HLA) class II region on chromosome 6 [11]. This region codes for the major histocompatibility complex (MHC) class II molecules, which function as antigen receptors on antigen presenting cells (APC). In celiac disease patients, gliadin peptides show high affinity to certain HLA heterodimers leading to a subsequent activation of T cells [12, 13].

The HLA heterodimer comprises of two ligands; the α-ligand and the β-ligand. The genes coding for these ligands reside in the HLA-DQ region in close linkage disequilibrium with the adjacent DR-region. Approximately 90-95% of celiac disease individuals carry the HLA-DQA1*05 and DQB1*02 alleles (coding for the molecule DQ2.5) [14] although carrying only one of these alleles seems to be sufficient for susceptibility to the disease [15]. These alleles can be situated in *cis* position (i.e. on the same chromosome): DR3-DQA1*05:01-DQB1*02:01 or in *trans* position (i.e. on opposite chromosomes): DR5-DQA1*05:05-DQB1*03:01/DR7-DQA1*02:01-DQB1*02:02 [16, 17] (**Figure 2**). The risk for celiac disease is increased if having two copies of the DQB1*02 allele, a so-called gene dosage effect [18], which is coupled to an increased T cell response [19] and have also been associated to changes in the phenotype [20-22]. The majority of the remaining 5-10% of individuals with celiac disease carry the haplotype DR4-DQA1*03:01-DQB1*03:02 (DQ8) and a minority of these carry the haplotype DR7-DQA1*02:01-DQB1*02:02 (DQ2.2) [23] (**Figure 2**).
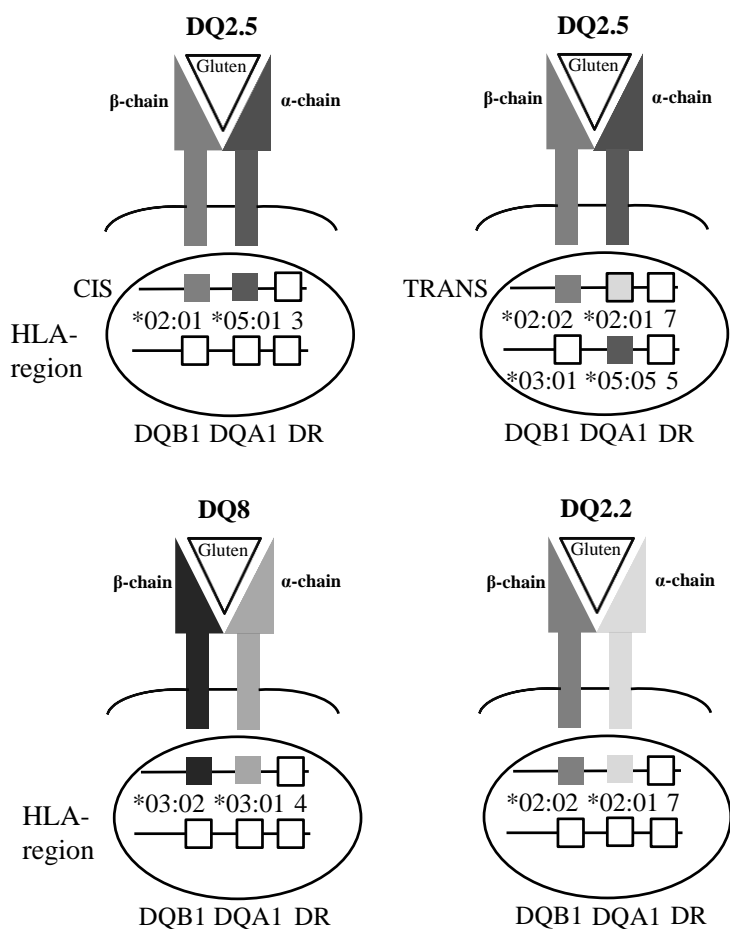
Figure 2. The HLA-haplotypes and DQ molecules associated with celiac disease. The HLA heterodimer, consisting of the α-chain and the β-chain, is coded for by alleles within the DQA1-DQB1-region. The α-chain coded by DQA1*0501 or DQA1*0505 differ by only one amino acid in the leader peptide and similarly, the β-chain coded by DQB1*0201 or DQB1*0202 differ by one amino acid in the membrane-proximal domain [17, 24]. Modified from an original figure in Sollid, L.M. Annu Rev Immunol, 2000. 18: 53-81.

Not having any of the aforementioned HLA haplotypes is considered a rarity among celiac disease individuals where the majority of non-DQ2.5 and non-DQ8 individuals carry half of the DQ2 heterodimer [15]. Few specific HLA alleles have been suggested to be protective for the development of celiac disease [25], such as HLA-DQB1*06:02 in type 1 diabetes (T1D) [26], but recently HLA-DPB1*04:01 was found to down-modulate the risk for having tTGA in DR3-DQ2 positive children [27].

Overall, genes within the HLA region are estimated to account for around 40% of the genetic predisposition in celiac disease and recently several loci within this region but outside the HLA-DQ region were identified [28, 29]. Genome wide association studies of single nucleotide polymorphisms have revealed 39 non HLA loci that could be of importance [30, 31]. Most of these loci involve the immune response and are shared with other autoimmune diseases [32].

Environmental factors

In Caucasians, approximately 30-40% are either DQ2 or DQ8 carriers [33]. Since the majority of these are gluten consumers but only a minority acquire celiac disease there has to be additional factors contributing to the disease risk [34] (**Figure 1**). The rapid change in incidence of celiac disease in very young children between different birth cohorts in Sweden during the mid-1980-90s that coincided with change in infant feeding recommendations of gluten clearly indicated that environmental exposures are important for the disease development [35, 36]. However, to date no single causal factor has yet been identified although efforts have been undertaken to study the association between first exposure to gluten and breastfeeding during infancy and subsequent risk for celiac disease [37-40]. Other factors proposed to be associated with celiac disease are pre- and perinatal events [41-43], infections[44, 45], drug use [46], nutritional defects [47], dysbiosis [48] and food processing [49].

Immune response to gluten

There are four major factors interacting in the pathophysiological process of celiac disease: gluten, the enzyme tissue transglutaminase type 2 (tTG), the HLA-DQ2 or DQ8 heterodimer molecules, and T cells [50]. In celiac disease patients, gliadin peptides pass the epithelial barrier of the small intestinal mucosa, probably trough mechanisms involving increased permeability [51], and reach the lamina propria in which they are bound to HLA-DQ2 and/or DQ8 heterodimer molecules on APC and presented to reactive T cells [52]. Activated T cells elicit an inflammatory response involving the release of the intracellular tTG which extracellularly catalyses the amino acid glutamine to glutamic acid by deamidation and render the gliadin peptides more negatively charged [53]. This enzymatic reaction increases the binding affinity of the antigen to the cleft of the HLA-DQ2 or DQ8 heterodimers stimulating a stronger gluten specific CD4+ T cell response [54]. These gliadin-specific, HLA-DQ2 or DQ8 restricted T cells are only found in celiac disease patients and not in healthy individuals [55]. The activation of CD4+ T cells in turn stimulate the development of T helper cells type 1 (T_H1), mediated by cytokines such as IFNγ, and the release of metalloproteinases by fibroblasts eliciting the typical histological injury in celiac disease characterized by increased

number of intraepithelial lymphocytes (IEL), crypt hyperplasia, and villus atrophy [56] (**Figure 3**).
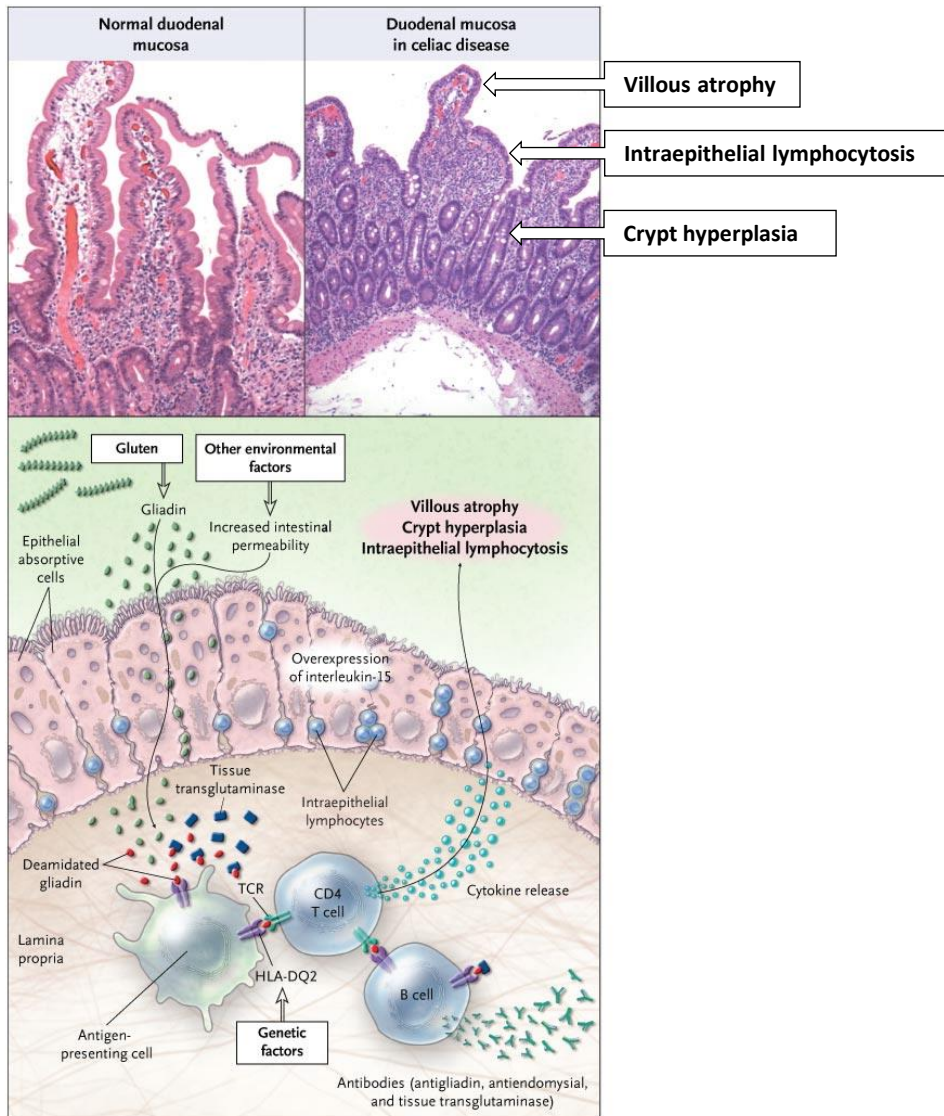
Figure 3. The immune response to gluten in celiac disease (bottom picture) and the most common histological mucosal changes (top picture). Clarifying block arrows added to original picture reproduced with permission from the publication of Green, P.H. et al. N Engl J Med, 2007. 357(17): 1731-43, Copyright Massachusetts Medical Society.

The role of the B cells in the pathophysiological process of celiac disease and its production of autoantibodies is still elusive [57]. The antibody production

dependency of gluten intake in addition to strong association to HLA-DQ2, DQ8 or both, indicates the involvement of gluten-specific T cells in the activation of different B cell clones and subsequent production of antibodies against cross-linked deamidated gliadin and tTG [16]. Once B cells are activated they in turn are proposed to activate additional CD4+ T cells leading to a vicious circle of enhanced activation and subsequent chronic inflammation [50].

Parallel to the adaptive immune response in the lamina propria CD3+ T cells in the epithelial lining of the intestinal mucosa are activated. Gliadin seems to be directly toxic by inducing production of IL-15 and other cell-surface ligands in enterocytes in an innate immune response [58]. This, in turn, activates natural killer cells involved in the destruction of the intestinal epithelium leading to villous atrophy [59, 60].

Cytokines in celiac disease

Cytokines are proteins secreted by cells of the immune system and serve as mediators of various functions ranging from stimulating growth and differentiation of lymphocytes, activating effector cells and stimulating the development of hematopoietic cells. Their main effect is exerted locally (i.e. autocrine or paracrine action), but if secreted in sufficient amounts they can reach the circulation and act on sites distant from the site of production (i.e. endocrine action). Cytokines mediate effects both within the innate and adaptive immune response characterized by activation of T cells as well as being signature cytokines of different subsets of T helper cells, e.g. T_H1 and T_H2 [61].

Several cytokines seem to be involved in the immunologic process in celiac disease. IL-15, produced by epithelial cells and dendritic cells, induces epithelial apoptosis by affecting IEL [62] and is suggested to make intestinal T cells unresponsive to suppressive effects of regulatory T cells [63]. IL-15 also induces the production of IL-21 from T cells and IEL which co-express T_H1 cytokines such as IFNγ [64]. IFNγ, the dominating cytokine produced by CD4+ T cells, induces the release of metalloproteinases by fibroblasts which degrade extracellular matrix and furthermore increase cytotoxicity of IEL [64]. IFNγ also enhances the HLA expression on APC thereby sustaining the response to gluten antigen [19]. Several studies have examined cytokine levels both locally in the intestinal mucosa [65, 66] as well as systemically in serum where they have been studied as markers of disease activity in affected individuals [67-71].

Prevalence

The prevalence of disease is defined as the proportion of a population having a certain condition e.g. a disease [72]. The prevalence can be measured at a certain time; point-prevalence, or during a period; period-prevalence. The prevalence varies depending on the population examined but also depending on method used for detecting affected individuals as well as definition of disease.

The prevalence of both screening-detected and clinically detected celiac disease in Sweden in 12-year old children has been estimated to between 2.2-2.9% depending on birth cohort studied [73]. In Norway, the prevalence of clinically detected celiac disease in 12-year old children or younger was estimated to 0.4% [74]. The prevalence of both clinically and screening-detected celiac disease in Finland in schoolchildren was 1% [75]. In contrast, the prevalence in Denmark is considered to be the lowest (0.08%) of the Scandinavian countries [76]. The prevalence of screening-detected and clinically detected celiac disease from other countries in Europe range from 0.3% [77] to 1.4% [78, 79] whereas prevalence in the United States is estimated to 0.8% [80]. In the non-western world celiac disease is believed to be as common as in Europe in the northern part of Africa, Middle-East and India, but is uncommon in China and adjacent countries [81]. All together, these prevalence studies have established the current belief that celiac disease is one of the most common chronic diseases both in adults and in children. To date, there is no plausible explanation to why Sweden has one of the highest reported prevalence numbers of celiac disease in the world.

Celiac disease is also known to be more prevalent in certain disease risk groups [82]. The largest risk group is first and second degree relatives of celiac disease individuals (prevalence of celiac disease 10-20%) [80]. Having another autoimmune disease, such as T1D (3-12%) [83], autoimmune thyroiditis (up to 7%) [84] or autoimmune liver disease (12-13%) [85], confers an increased risk to develop celiac disease compared to the general population. Also, having IgA deficiency (2-8%) [86], Downs's syndrome (5-12%) [87], Turner syndrome (2-5%) [88] and William's syndrome (up to 9%) [89] are linked to the comorbidity of celiac disease. This increased risk has led to the recommendation of routine screening among these groups of patients in the clinical setting [82].

In individuals carrying the HLA-DQ risk alleles, longitudinal prospective population studies that follow at genetic risk birth cohorts selected from the general population have clearly shown that the incidence of celiac disease varies depending on HLA genotype. The DAISY study (Diabetes and Autoimmunity Study in the Young) from Denver in Colorado, United States, showed that the cumulative incidence of celiac disease in children carrying HLA-DR3-DQ2/X (i.e. heterozygotes for DQ2) was 3.4% by 5 years of age [90]. In the DIPP study

(Diabetes Prediction and Prevention) from Finland, following children with HLA-DQB1*02, the prevalence of celiac disease was 1.3% by mean 5 years of age [91]. The multicentre study TEDDY (The Environmental Determinants of Diabetes in the Youth), involving six clinical centres in four different countries, has revealed that the incidence of celiac disease is dependent on HLA-genotype in young children [22]. However, none of these prospective studies have enrolled children having HLA alleles not associated to celiac disease and thus the prevalence of celiac disease in this HLA-non-risk group is not known. The prevalence of celiac disease thus varies substantially between geographical regions and different subpopulations, which emphasises that both genetic and environmental factors are important for its widespread occurrence. More importantly, the reported prevalence also seems to be dependent on whether screening of the general population has been performed or not highlighting the importance of method for identification of disease.

Clinical Presentation

Celiac disease is associated to a myriad of symptoms and manifestations, which range from classical gastrointestinal symptoms including malabsorption to more subtle variants. The description of the disease has changed over time, possibly because of increased knowledge about disease manifestations, but probably also because of true changes in the clinical pattern towards milder forms and more often extra-intestinal symptoms and older age at diagnosis [36, 92-94]. Classically, in young children (< 3 years), symptoms often include intestinal symptoms like diarrhoea and abdominal distension but also extra-intestinal symptoms like growth retardation. Older children (≤18 years) often present with constipation, abdominal pain and extra-intestinal manifestations such as fatigue, delayed puberty, and arthralgia [95]. The extra-intestinal symptoms like iron deficiency anaemia, elevated liver enzymes, skin lesions of dermatitis herpetiformis, neurological manifestations such as neuropathy, and psychiatric disorders like depression needs knowledgeable medical personnel to be identified as symptoms of celiac disease [96].

More importantly, many celiac disease patients can be virtually *asymptomatic* (i.e. lack of symptoms even in response to direct questioning at diagnosis), or have *subclinical* forms (i.e. having insufficient symptoms to raise the suspicion of celiac disease in clinical care) [97]. In fact, prospective birth cohort studies have revealed that the majority of children with celiac disease that are identified by screening only exhibit mild symptoms [98, 99] and would most certainly be unrecognized without the screening procedure [100]. Thus, the understanding of

celiac disease presentation to date points in the direction of symptoms not being reliable tools for identifying disease and other means of recognition is warranted.

Diagnosis

The diagnostic work-up for celiac disease of today constitutes of a combined arsenal of disease specific serological markers, histology and genetic tests.

Serology

Since the 1980s, the first step towards a celiac disease diagnosis has been the analysis of presence of autoantibodies in serum and today, one of the most widespread applied antibody tests is the detection of IgA autoantibodies directed against tissue transglutaminase (tTGA) [3]. Different methods for detection have yielded high sensitivity between 90-95% and specificity around 99-100% [101]. Autoantibodies are analysed using either solid phase enzyme linked immunosorbent assays (ELISA) or liquid phase radioligand binding assays (RBA) and the two methods have shown a good correlation [102].

Autoantibodies directed against the endomysium, IgA-EMA, were discovered before tTGA and have also yielded high sensitivity and specificity above 90% for celiac disease [103]. IgA-EMA is analysed by indirect immunofluorescence, which is considered expensive and associated with inter observer variability not found in ELISA or RBA.

IgG antibodies against deamidated gliadin peptides (DGP), developed the last 15 years, have comparable sensitivity and specificity as IgA-tTGA [103] and in the revised ESPGHAN diagnostic criteria for celiac disease, IgG-DGP is recommended to be used in patients with IgA deficiency and in children younger than 2 years of age [82]. As both IgA-tTG and IgA-EMA display a lower sensitivity in children younger than 2 years of age compared to older children, IgA anti-gliadin antibodies (AGA) could be an option, but should be interpreted with caution because of its low specificity [104] and in a recent study IgA-tTGA showed sufficient sensitivity even in very young children [105]. Since celiac disease is more common in IgA deficient individuals, it is important that all IgA based tests are accompanied by measurements of IgA levels in serum [106]. In IgA deficient celiac disease individuals, IgG-tTGA and IgG-DGP have shown comparable sensitivities as for IgA-tTG in IgA-sufficient cases [107].

Finally, gluten consumption is a prerequisite for reliability of detection of all of these aforementioned antibodies thus the tests can also be used as markers for response to treatment and dietary compliance.

Histology

The assessment of duodenal biopsies has long been considered the gold standard of celiac disease diagnostics. During the past decades, the perception of celiac disease being an autoimmune disease not just affecting the intestinal mucosa has questioned this belief. The discovery of disease specific autoantibodies and the development of reliable methods have led to the recommendation that histological diagnosis can be omitted among children in clear cut cases with high autoantibody levels [82]. However, in unclear cases with low levels of tTGA and subtle symptoms or no clinical signs of the disease, intestinal biopsies taken via upper endoscopy or via capsule still offers an important diagnostic approach. On the contrary, histological diagnosis is still recommended for all adults due to e.g. differential diagnoses such as malignancies or refractory disease [108]. It should be kept in mind that the affected intestinal mucosa in celiac disease patients may be patchy [109], why it is recommended to take multiple biopsies from the duodenum as well as from the bulb [110].

The typical microscopic histological alterations in celiac disease include infiltration of plasma cells and lymphocytes in the lamina propria, increased numbers of IEL, elongated crypts, and partial or total villous atrophy [111] (**Figure 3**). These histological findings are classified according to the Marsh-Oberhuber classification in which the normal mucosa is denoted Marsh 0. The infiltrative lesion is defined as a normal mucosal architecture but increased number of IEL; > 40 IEL/100 enterocytes called Marsh 1. In the hyperplastic lesion there is still a normal villous architecture but an increased number of IEL in combination with crypt hyperplasia which is called Marsh 2. In the next stage, called the destructive lesion, increased IEL, crypt hyperplasia and various degrees of villous atrophy are present (partial villous atrophy- Marsh 3A, subtotal villous atrophy- Marsh 3B and total villous atrophy- Marsh 3C). The hypoplastic lesion, called Marsh 4, is defined as a flat mucosa with normal count of IEL and normal crypts [112, 113]. It is important to notice that the histological alterations are not pathognomonic of celiac disease and can be found in other conditions such as cow's milk protein allergy, giardiasis, Crohn's disease, inherited enteropathies, and drug reactions [111]. Additional diagnostic histological markers are therefore being developed such as count of IEL at the villous tip, $\gamma\delta+$ IEL, and intestinal deposits of tTGA [114, 115]. Finally, the inter-observer variability is important to consider when histological specimens are being interpreted.

Genetic testing

The strong negative predictive value of HLA-DQ2, DQ8, or both, for celiac disease makes genotyping an applicable method for selecting individuals at genetic risk. HLA genotyping can be assessed by polymerase chain reaction (PCR)

and hybridization with allele specific probes; a highly specific but time consuming and expensive method. However, the positive predictive value is low as 30-40% of populations in the Western World (Caucasian, White or European origin) are carriers of HLA-DQ2 and/or DQ8 haplotypes [33, 116] but only about 1% develop celiac disease. Thus, the clinical value of testing for HLA-DQ risk alleles has mostly been in the case of excluding celiac disease in dubious cases [117]. Alternative methods for detection of HLA-risk alleles in celiac disease such as single nucleotide polymorphisms (SNPs) are currently being developed [118, 119], but so far testing for non-HLA genetic risk loci has only been done in the research setting [120].

Diagnostic criteria in symptomatic celiac disease

The first diagnostic criteria for celiac disease, established by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) in 1969, recommended the use of intestinal biopsies as the gold standard of methods to confirm villous atrophy at diagnosis, mucosal healing after gluten-free diet and relapse of the disease after gluten challenge. After several reviews, the ESPGHAN criteria were revised in 1990 to include only one initial diagnostic biopsy and thereafter a clear cut response to gluten-free diet with normalisation of serological markers as combined criteria [121]. With increased experience of the high diagnostic performance of tTGA and EMA, ESPGHAN revised the guidelines in 2012 and accepted a tTGA level $\geq$ x10 the upper limit of normal confirmed by EMA and HLA as a replacement for the biopsy as the gold standard [82]. Still, an intestinal biopsy is recommended in dubious cases and tTGA levels should seroconvert to normal on a gluten-free diet [82].

Diagnostics in screening-detected celiac disease

In asymptomatic children at genetic risk, tTGAs are considered reliable tools for identifying celiac disease [122], but low levels of tTGA are more common than in symptomatic children [123]. The ESPGHAN guidelines therefore recommend HLA genotyping as the first step followed by tTGA as a second test in screening individuals at increased risk for celiac disease, such as first-degree relatives of celiac disease individuals, T1D patients, individuals with IgA deficiency, and Down syndrome [82]. In first-degree relatives and children with T1D there is a high rate of normalisation of tTGA despite a gluten containing diet [124, 125]. In addition, asymptomatic children detected with elevated levels of tTGA are recommended a duodenal biopsy to confirm diagnosis [82].

Follow-up of childhood celiac disease should include evaluation of symptoms and check-up of tTGA to control response to treatment and dietary compliance, but a control biopsy is not routinely recommended [82]. The proportion of mucosal

recovery in children is considered to be high, but there is up to date limited evidence in children to support any of the applied follow-up measurements although serology seems to have a high negative predictive value [126].

Screening

Medical screening consists of three elements. First, it is a process of selection with the purpose of identifying those individuals who are at a sufficiently high risk of a specific disorder to warrant further investigation or sometimes direct preventive action. Second, screening should be systematically offered to a population who would not seek medical attention on account of symptoms of the disease for which screening is being conducted. Third, the purpose of identifying a disease should benefit the individual [127].

In the 1960s, Wilson and Jungner published for the World Health Organization (WHO) ten criteria to be fulfilled for a disease to be considered for mass screening [128]:

1. The condition sought should be an important health problem.

2. There should be an accepted treatment for patients with recognized disease.

3. Facilities for diagnosis and treatment should be available.

4. There should be a recognizable latent or early symptomatic stage.

5. There should be a suitable test or examination.

6. The test should be acceptable to the population.

7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.

8. There should be an agreed policy on whom to treat as patients.

9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.

10. Case-finding should be a continuing process and not a "once and for all" project.

These criteria have been modified since then by different health organisations in various countries but they still remain the basis when mass screening is considered.

Screening is a method of prevention, i.e. any activity that reduces the burden of mortality or morbidity from disease. Primary prevention is aimed at preventing the development of disease, in celiac disease by inducing tolerance to gluten, which to date is not possible, but much aim is focused on if early infant feeding could be optimized and intervention by vaccination in risk individuals [129]. On the contrary, screening for celiac disease could be beneficial at a secondary as well as at a tertiary prevention level. As secondary prevention, identification of individuals with unrecognized disease, possibly at an early stage, increases the opportunities for interventions to halt disease progression and the emergence of symptoms. As tertiary prevention, identifying undiagnosed individuals and instituting a gluten-free diet will reduce the negative impact of an already existing disease by restoring function and reducing disease-related complications.

Mass screening for celiac disease

Most of the screening criteria are fulfilled for symptomatic celiac disease, whereas in subclinical and asymptomatic celiac disease the benefits of diagnosis and treatment are still a matter of debate [129-132].

First, it has not yet been fully clarified whether having asymptomatic celiac disease confers a health problem to the individual (i.e. criteria 1). An argument for early identification is the finding of low bone mineral density in young children with subclinical or potential celiac disease [133]. In addition, young children found in screenings often display symptoms albeit they are mild [98, 99]. On the other hand, health-related quality of life (HRQoL) prior to diagnosis seems not to be affected [134].

Second, performing an intestinal biopsy during anaesthesia in tTGA positive individuals is an invasive procedure and expensive method associated with risks, which could be questioned in asymptomatic children (i.e. criteria 6), although a recent study has demonstrated that affected children seems to be positive towards screening in general [135].

Third, there is a lack of information on the health benefits of treatment in screening-detected celiac disease and potential negative effects of a restricted diet and reduced compliance (i.e. criteria 8). HRQoL, symptom reduction and compliance after starting a gluten-free diet show overall positive results in children detected by screening [136-140] as well as in adults [141]. Although studies on mortality have not yet examined screening-detected celiac disease in children [142, 143], the mortality in adults with celiac disease detected by screening seemed not to be affected [144].

Fourth, screening will probably identify a number of individuals with celiac disease autoimmunity (i.e. positive tTGA) or potential celiac disease (i.e. tTGA positive with normal mucosal features). The long-term risk of complications in

children with celiac disease autoimmunity with no or only mild histological alterations have not been fully explored (i.e. criteria 7 and 8). Several studies point in the direction that a large fraction of children with celiac disease autoimmunity and potential celiac disease lose their tTGA or do not develop mucosal changes at follow-up and therefore screening could create unnecessary concern [91, 125, 145, 146]. Mild histological alterations have so far not been considered diagnostic for symptomatic celiac disease but there are studies on both children and adults in favour of treatment [143, 147-149].

Finally, there is a lack of cost benefit studies of mass screening in children and how a screening procedure could be implemented in the general health care system still needs to be established (i.e. criteria 9) [150, 151].

Treatment

To date, the only recommended treatment for celiac disease is a strict gluten-free diet, i.e. a diet free from wheat, rye and barley, which is considered a safe and harmless treatment with nutritional and energy contents comparable to a normal diet [152]. Non-adherence is coupled to certain risk-factors such as young age at diagnosis but does not appear to be related to symptoms at diagnosis [153] and compliance in patients with celiac disease detected by screening seems to be high [137, 138]. The social burden of a gluten-free diet should not be overlooked, especially during adolescence when dietary advice should be modified accordingly. Based on this assumption, alternative therapies are being developed. Glutenases that enzymatically degrade gluten to non-toxic peptides, polymers that bind gluten intra-luminally, tight junction modulators such as zonulin-antagonists, and blocking of lymphocyte homing in the intestines, are potential drugs currently under development [154].

Associated diseases and long-term complications

Celiac disease is associated with other diseases and long-term complications later in adulthood if left untreated. Hitherto, the associated risks have been assessed in studies of individuals with clinically diagnosed celiac disease whereas the risks in individuals with subclinical and screening-detected disease are unresolved.

The overall risk for an individual with celiac disease to develop another autoimmune disease is increased affecting 15-20% of celiac disease patients [155] of which thyroiditis is the most prevalent (3-10%) [156]. In addition, the risk for T1D in celiac disease individuals is more than twice that of the general population [157] and the prevalence of celiac disease in T1D patients is approximately 6%

[83]. The reasons for the co-occurrence of autoimmune diseases is unknown but is probably mainly due to the shared HLA haplotypes and possibly other identified risk genes outside the HLA region [158]. However, it has been speculated whether gluten may be a trigger also for T1D, either as a triggering antigen on the pancreatic islet beta cells or indirectly by causing an increased intestinal permeability for other antigens such as enteroviruses [159], but whether the risk of other autoimmune diseases in celiac disease is modulated by a gluten-free diet remains unclear [160, 161].

The probably most feared of complications is the risk for malignancies, which have been extensively studied in patient register studies [162-164]. Celiac disease individuals have a slight increased risk for malignancies, preferentially during the first year after diagnosis after which the risk levels off comparable to the general population [165]. Malignancies associated with celiac disease are non-Hodgkin lymphoma, especially enteropathy-associated T-cell lymphoma (EATL), which in turn is closely linked to refractory celiac disease type 2, and gastrointestinal adenocarcinoma [166]. However, these are rare diseases preferably presented in adults and the absolute risks in celiac disease individuals are low [166]. Also, mortality risk is increased in both adults as well as children with celiac disease, but decreases by time after diagnosis [143].

Other long-term potentially severe complications are pregnancy related complications which have been found to be more common in untreated women with celiac disease in contrast to treated individuals in some [167], albeit not in all studies [168, 169]. Infertility has been discussed as a long-term complication of celiac disease, but register based studies from Sweden do not support this [170, 171].

In contrast to the aforementioned complications, the risk of osteopenia or osteoporosis and related fractures in celiac disease is far more common [172, 173]. In a study on middle-aged women selected from the general population, high levels of tTGA were associated with lower bone mineral density (BMD) and higher fracture frequency in women between 50 and 64 years of age [174]. More importantly, a gluten-free diet improves BMD in adults within a year although not all individuals normalize their bone mass at long-term follow-up [175, 176]. The risk of low BMD and osteoporosis have also been studied in children and adolescents with celiac disease, but there are some circumstances that needs to be considered when it comes to the study of bone mass during childhood.

Bone mass during childhood

The skeleton accumulates bone mineral up till the end of the second or the beginning of the third decade of life to reach its peak bone mass (PBM); the highest bone mass value in life [177]. Once the PBM reaches its plateau the bone

30

mass starts to decline which accelerates with increasing age, particularly in women during the 10 year period after menopause [178]. The most rapid accrual of bone mineral occurs in girls between 11-14 years of age and in boys between 13-17 years of age, respectively [179]. PBM is affected by different life-style factors; physical activity, nutrition, chronic diseases, but the most important regulator are hereditary factors [177, 180] (**Figure 4**).
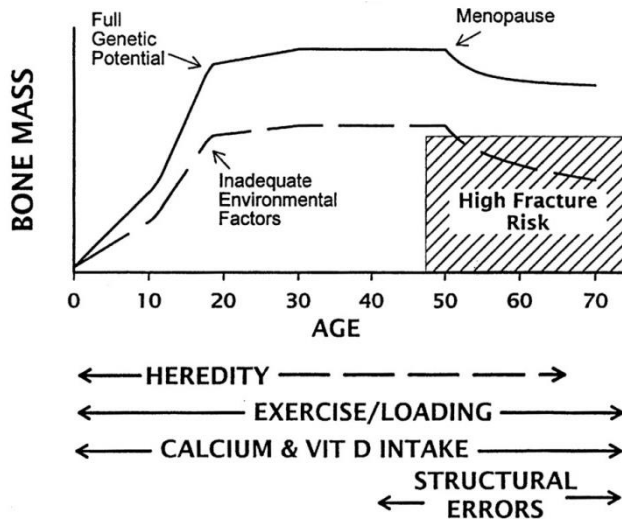
Figure 4. Bone mass acquisition and changes during life and factors affecting outcome. Reprinted from Heaney et al., Osteoporos Int, 2000. 11(12): 985-1009 with permission from the publisher.

PBM is an important predictor of osteoporosis later in life and adult decrease in BMD increases risk for fractures [181, 182]. Children affected by a chronic disease during growth, such as inflammatory bowel disease, are at an increased risk to gain a lower PBM thus affecting BMD both during childhood and adult life [183]. However, it is important to underline that a low BMD does not solely cause fractures and that fracture risk is affected by several other factors that can coincide with low BMD, such as low muscle strength. It is also important to know that bone tissue is under constant turnover throughout life, a process called remodelling [184].

Bone mass measurement

Bone mass is often expressed as bone mineral content (BMC) (g), which refers to the mineral detected at measurement or areal BMD (g/cm^2),which is bone mineral detected over a projected area. There are several methods to assess BMC and BMD of which dual X-ray absorptiometry (DXA) is considered the gold standard for bone measurement [185]. This technique is based on filtered X-rays of two

energies, which enables the distinction between soft tissue and bone, and a detector on the other side of the measured individual that can be used to calculate bone mass. Compared to other methods it can scan all parts of the body, it is a fairly rapid procedure (a total body scan of a 10-year old child takes approximately 7 minutes), it produces a low radiation dose (1-8 μSv per scan corresponding to 1/1000 of the yearly background radiation) and combines a high accuracy (the closeness of a measurement to the true value) as well as a high precision (the reproducibility of a measurement when repeated) [186].

Osteoporosis in children is defined as the presence of a clinically significant fracture history and low BMC or BMD. In children, a low BMC/BMD, defined as a Z-score (number of SD from the mean of a population adjusted for age and gender) < -2.0, should be used instead of the term osteopenia [187]. If osteoporosis is related to another disease or medication it is often referred to as secondary osteoporosis [188].

BMD in children with celiac disease

Children with celiac disease have reduced BMC and BMD at diagnosis compared to healthy individuals [189, 190]. A gluten-free diet increases BMD to normal levels within a year of treatment in some previous studies [191-194], and BMD is maintained on a long-term follow-up [195, 196], whereas other studies have shown lack of normalisation after 1 year [197] and 2 years of treatment, respectively [198]. There is also some evidence that children who are older at diagnosis have lower BMD adjusted for age compared to younger children indicating that age at diagnosis influence outcome of BMD [199-203]. The dietary compliance has also been evaluated in favour of a strict gluten-free diet [204, 205]. Neither an association between presence of clinical symptoms and BMD have been found [201, 206], nor between BMD and levels of tTGA, histological alterations or laboratory measurements such as parathyroid hormone (PTH) [198, 206].

Studies on biomarkers of bone-formation and bone-resorption have shown an increased activity of bone-resorption markers and decreased activity in bone-formation markers at diagnosis, which is reversed after start of a gluten-free diet [194, 207]. The majority of studies do not demonstrate changes in vitamin D metabolites or in PTH levels in children with untreated celiac disease [195, 200, 208]. Fracture risk in children with celiac disease is not well-studied, although the absolute risk of associated hip fractures in childhood celiac disease seems to be low [209].

However, many of these previous studies are performed on clinically detected celiac disease. In adults, studies on patients with celiac diseases detected by screening [210] as well as asymptomatic individuals with a former celiac disease

diagnosis but on a gluten containing diet [211, 212], all demonstrate the presence of osteopenia/osteoporosis in the majority of patients. Only one prospective cohort study of screening-detected tTGA positive asymptomatic preschool children have been performed showing a lower BMD in screening-detected subjects compared to tTGA negative controls [133]. This highlights the need for additional studies of BMD in children identified in screenings.

Causes of low BMD in childhood celiac disease

There are several possible factors that can interact to alter bone metabolism in celiac disease but there is up to date no clear evidence for a specific main cause [213] (**Figure 5**).
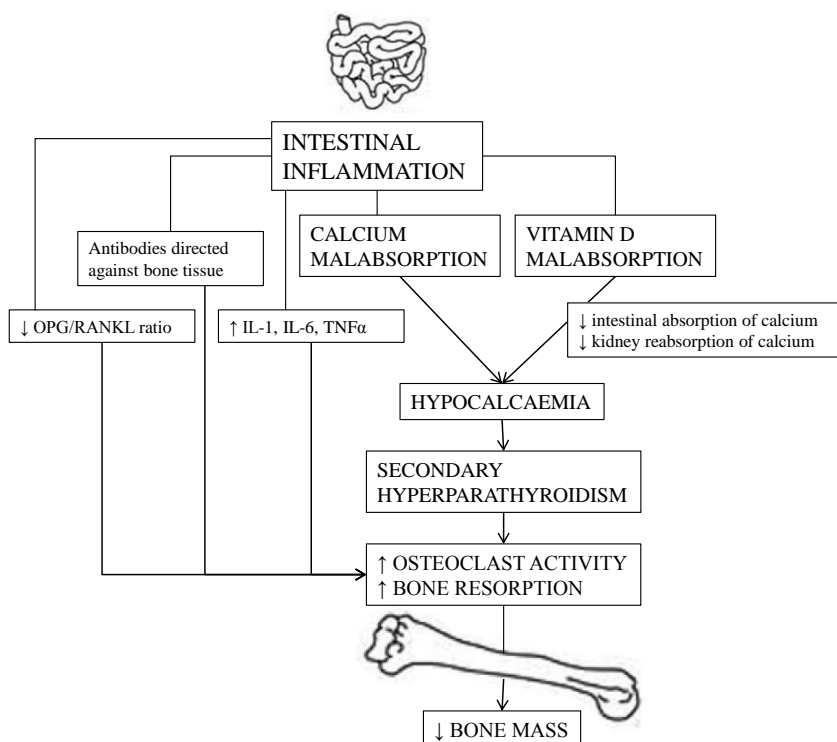
Figure 5. Possible mechanisms involved in bone loss in celiac disease.

Malabsorption of calcium and vitamin D in the duodenum and jejunum and secondary hyperparathyroidism is one possible cause for increased bone resorption and decreased formation shown in adults in contrast to children [200, 214]. Theoretically, malnutrition of these nutrients as a result of secondary lactose intolerance found in celiac disease could be another possibility, but is probably of

less importance in countries where lactose-free products are calcium and vitamin D enriched and easily accessible. Severe disease with malnutrition and fatigue leading to reduced physical activity in combination with reduced sun exposure resulting in vitamin D deficiency could in theory contribute to low BMD.

Inflammation derived cytokines have long been known to alter bone formation, leading to bone loss in chronic paediatric inflammatory conditions such as in inflammatory bowel disease [215], rheumatologic diseases [216] as well as in celiac disease. The cytokines IL-1β, IL-6 and TNF-α are able to activate osteoclasts (i.e. bone cells in charge of bone resorption) and have been found elevated in adults with untreated celiac disease [217]. In synergy, IL-12 and IL-18 associated to inhibition of osteoclasts have been found to be down-regulated in adult celiac disease individuals [218]. Most cytokines are thought to regulate bone remodelling directly or indirectly through the RANK/RANKL/OPG pathway [219]. Receptor activator nuclear factor κB (RANK) is located on pre-osteoclasts. When receptor activator nuclear factor κB ligand (RANKL) is bound to RANK this stimulates the differentiation and activation of osteoclasts, which promotes bone resorption. Osteoprotegrin (OPG) acts as a decoy receptor for RANKL and inhibits RANK/RANKL interaction and therefore inhibits osteoclast activity. Both RANKL and OPG are secreted by osteoblasts (i.e. bone cells responsible for bone formation) as well as by other tissue cells. The OPG/RANKL ratio has been found to be lower in celiac disease patients compared to healthy individuals, implicating its role in bone loss in celiac disease [218, 220].

Autoantibodies produced as a part of the inflammatory process in celiac disease have also been postulated to play a role in bone loss. Sera from untreated celiac disease individuals provide evidence of presence of IgA autoantibodies directed against bone specific tTG, which is an enzyme important for bone matrix stabilisation and mineralisation [221]. Autoantibodies directed against OPG have been found in celiac disease patients associated with lower BMD [222, 223], but the clinical significance of testing for these antibodies are still contradicting [224].

Despite the body of evidence that BMD is affected also in children with celiac disease, there is no consensus whether DXA should be performed as a standard procedure on a regular basis. Furthermore, there is no other treatment except for gluten-free diet recommended in children with celiac disease [225].

Aims

The overall aim of the research and the basis for this thesis was to identify children with screening-detected celiac disease in an HLA-genotyped birth cohort and to study subclinical manifestations of the disease.

The specific aims were:

I.   To estimate the prevalence of screening-detected celiac disease in an HLA-genotyped cohort of children at 3 years of age (Paper I).

II.   To estimate the prevalence of screening-detected celiac disease in a follow-up study of a cohort of HLA-genotyped children at 9 years of age (Paper II).

III.   To examine signs of systemic inflammation by measuring cytokines in serum of 3-year old children with screening-detected celiac disease at time of diagnosis and after treatment with a gluten-free diet (Paper III).

IV.   To assess if 9-year-old children with screening-detected celiac disease have affected bone mineral density (BMD) and/or levels of 25(OH) vitamin D3 and parathyroid hormone (PTH) at time of diagnosis and under treatment with a gluten-free diet (Paper IV).

Methods

Population and study design

This thesis is based on the Celiac Disease Prediction in Skåne (CiPiS) study; a prospective population-based cohort study aimed at identifying celiac disease by screening in children born in the region of Skåne 2001-2004. Skåne is a province situated in the southern part of Sweden with 1.2 million inhabitants (2004) in which approximately 12.000 children were born yearly during the time period 2001-2004 (www.scb.se) (**Figure 6**).
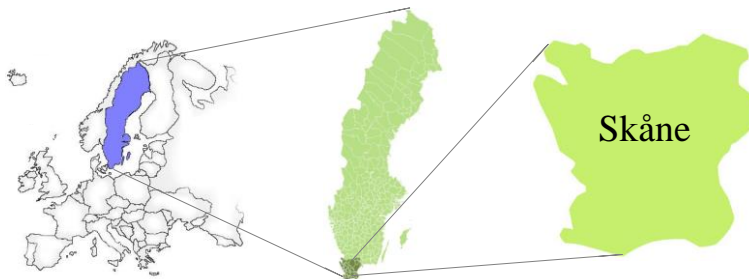
Figure 6. The Celiac Disease Prediction in Skåne (CiPiS) study is based on a cohort of children born in the region of Skåne, Sweden 2001-2004.

DiPiS study

The CiPiS study is part of the DiPiS (Diabetes Prediction in Skåne) study which is a prospective cohort study aimed at determining the predictive value of genetic risk in combination with diabetes autoantibodies and to identify non-genetic risk factors for T1D in all children born in Skåne during the years 2000-2004 [226].

During the study period, all parents of newborn children were invited to participate in the DiPiS study at delivery, and after oral consent, cord blood was collected for HLA genotyping, analysis of islet autoantibodies, and a registration form was filled in. The form contained information about the mother's age, date of birth, multiple births, gestational age, gender, diabetes in mother, gestational diabetes, and maternity clinic. When the child was two months of age, parents were sent a

letter of invitation to continue participation and participating parents gave their written informed consent and filled in questionnaires concerning hereditability for diabetes and factors relevant to diabetes during pregnancy, delivery and the first two months of the child's life [226, 227]. Parents of participating children who had increased risk for developing T1D were contacted again when the child was two years of age for continued participation and a blood sample was drawn from the child and sent in for analysis of islet autoantibodies and thereafter annually. The increased risk for T1D was mainly based on the HLA genotype of the child, but other risk factors was also taken into account (heredity for diabetes, infections during pregnancy, mothers age >40 years, islet autoantibodies in cord blood, large for gestational age and postnatal jaundice).

CiPiS study

All children born between June 2001 and August 2004 whose parents answered the two-month questionnaire in DiPiS were eligible for participation in the CiPiS study. According to Statistics Sweden (www.scb.se) approximately 39,000 children were born in Skåne during this period. A DiPiS-registration form at delivery was received from 29,913 newborns and no information was obtained from the approximately 9000 remaining newborn children. Successful HLA genotyping was available from 29,039 children and parents of 19,621 children gave written consent to continue participation at two months (**Figure 7**). All children having either HLA-DQB1*02 or *03:02, or both alleles, were considered having genetic risk for celiac disease. Children having HLA-DQA1*05:01, also conferring risk for celiac disease, were not all identified since only children having HLA-DQB1*02 were HLA-DQA1-genotyped (see paragraph about HLA-DQ genotyping) [15, 228]. The HLA-DQB1 distribution of the genotyped population, of children to parents accepting participation in DiPiS study (answering the questionnaire), and of children invited to CiPiS study is shown in **Table 1**. During the study period, the HLA-DQB1*02 and DQB1*03:02 alleles occurred in 56.8% of the HLA-genotyped population comprising of more than 70% of the children born in the studied region.

At 3 years of age, two groups were invited to participate in the CiPiS study. Children having either HLA-DQB1*02, DQB1*03:02, or both alleles, were included in the HLA–risk group. Children not carrying any of these risk alleles were included in the HLA-non-risk group. The HLA-risk group were recruited mainly from the DiPiS invited group constituting of 90% of children having the high risk HLA-alleles for T1D and 40% of children having neutral risk for T1D (**Table 1**). In addition, 15% of the children carrying a protective allele for T1D were also invited to the CiPiS study (**Table 1**). Children without any risk alleles were invited regardless of if they were invited to the DiPiS study. In all, 13,860

children were invited at 3 years of age of whom 6206 children belonged to the HLA-risk group and 7654 children to HLA-non-risk group (**Figure 7**).

At 9 years of age, the same group of children were re-invited to a follow-up screening (**Table 1**). All children who had been diagnosed with screening-detected celiac disease at 3 years of age, those who had moved, died or denied continued participation, were excluded. Altogether, 13,024 children were invited in the follow-up screening at nine years of age of whom 5947 belonged to the HLA-risk group and 7077 to the HLA-non-risk group (**Figure 7**).
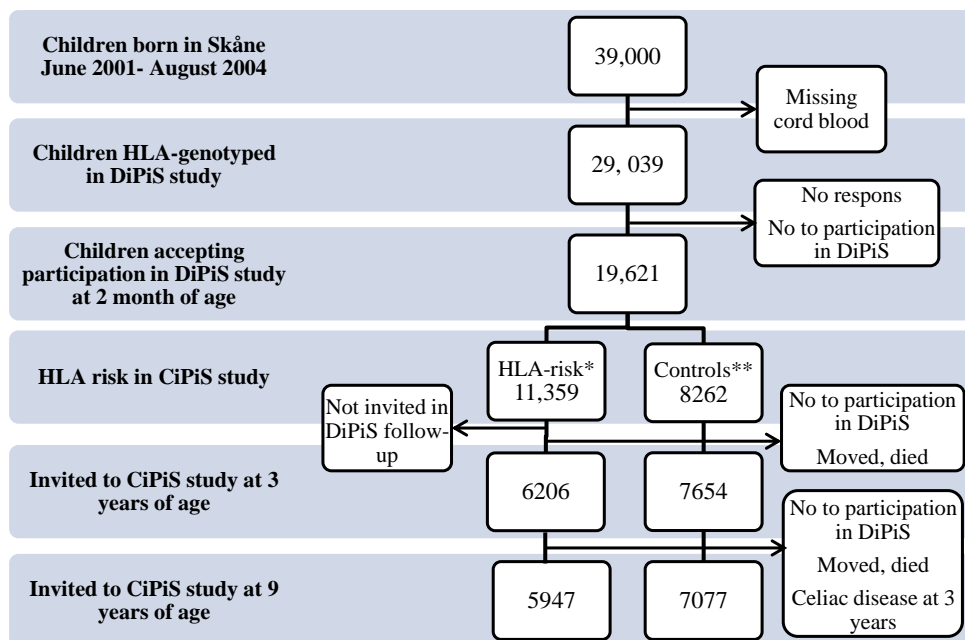
Figure 7. Children accepting participation in DiPiS (Diabetes Prediction in Skåne) study and children invited to CiPiS (Celiac Disease Prediction in Skåne) study. *HLA risk in CiPiS is defined as having HLA-DQB1*02 and/or *03:02 alleles. ** Controls are HLA-non-risk group defined as children not having HLA-DQB1*02 and/or *03:02 alleles.

Parents of invited children were sent an information letter and were asked to respond regardless of they wanted to participate or not. They were also asked to respond if the child already had celiac disease and these children were excluded from the screening. In the screening at 9 years of age, non-responding parents were sent a letter of reminder and if still not responding they were reminded by phone call from a study nurse. Parents (one parent in the first screening and, if possible, both parents in the follow-up) of participating children gave their written consent and if so, they were sent a tube for collection of a blood sample at their local health care centre. In the screening at 9 years, they were also offered to have

the blood sample drawn by the school nurse, at the research centre or by the parent following self-instructions. After collection, the blood samples were sent by mail to the research centre.

In participating children, i. e. in those children in which parents gave written consent and a blood sample was sent to the research centre, blood samples were analysed for tTGA. Children with a positive result were contacted by phone call by a paediatrician and study investigator and offered a follow-up blood sample after 3 months. The follow-up sample was collected in the same manner as the first sample. Regardless of the result of tTGA analysis in the second sample, the parents were again contacted by the study investigator for information about the study result. Children with a second positive result with at least one value above borderline zones (see paragraph about Tissue transglutaminase autoantibody analysis) in any of the two consecutive samples were defined as persistently tTGA positive and qualified for intestinal biopsy. Parents to children accepting continued investigation by intestinal biopsy in the screening at 3 years were referred to their local paediatric clinic for intestinal biopsy. In the screening at 9 years of age, all children were referred to the Department of Pediatrics at Skåne University Hospital in Malmö, Sweden, for investigation with intestinal biopsy. All children, regardless of biopsy result, were referred to their local paediatric clinic for follow-up after investigation.

At 9 years of age, all children accepting continued investigation with intestinal biopsy were also examined by DXA, anthropometric measurements and additional blood samples were collected at the same appointment as the gastroscopy. Matched controls and children diagnosed with screening-detected celiac disease from the first screening were also invited by phone call from a paediatrician and study investigator or a study nurse for the same measurements excluding intestinal biopsy.

Table 1. HLA-DQB1 genotypes of children born in Skåne June 2001-August 2004 (comprising of approximately 70% of total population) and of children participating in Diabetes Prediction in Skåne (DiPiS) study at 2 months of age as well as HLA-DQB1 genotypes of children invited to Celiac Disease Prediction in Skåne (CiPiS) study at 3 years of age and at 9 years of age.

HLA risk for T1D in DiPiS	HLA-DQB1 genotype		HLA-genotyped population	Answered DiPiS questionnaire at 2 months of age	HLA-risk for Celiac Disease[#]	Invited to CiPiS at 3 years who answered the questionnaire at 2 months of age	Invited to CiPiS at 9 years who answered the questionnaire at 2 months of age
	Allele 1	Allele 2	N (%)	N (%)		N (%)	N (%)
High risk	*02[a]	*X	2048 (7.1)	1383 (67.5)	Yes	1278 (92.4)	1210 (87.5)
	*02[a]	*03:02	1059 (3.6)	762 (72.0)	Yes	699 (91.7)	697 (91.5)
	*02[a]	*06:04	403 (1.4)	290 (72.0)	Yes	275 (94.8)	266 (91.7)
	*03:02	*X	2381 (8.2)	1604 (67.4)	Yes	1481 (92.3)	1454 (90.6)
	*03:02	*0604	381 (1.3)	275 (72.2)	Yes	256 (93.1)	255 (92.7)
Neutral risk	*02[b]	*03:02	570 (2.0)	374 (65.6)	Yes	162 (43.3)	151 (40.4)
	*02[b]	*06:04	191 (0.7)	142 (74.3)	Yes	63 (44.4)	57 (40.1)
	*02[b]	*X/*02[a]	1888 (6.5)	1268 (67.2)	Yes	530 (41.8)	493 (38.9)
	*02[c]	*03:01	1977 (6.8)	1324 (67.0)	Yes	562 (42.4)	534 (40.3)
	*03:02	*03:01	1360 (4.7)	916 (67.4)	Yes	370 (40.4)	350 (38.2)
Low risk	*02[c]	*06:02/*06:03	2548 (8.8)	1783 (70.0)	Yes	292 (16.4)	257 (14.4)
	*03:02	*06:02/*06:03	1702 (5.8)	1238 (72.3)	Yes	239 (19.3)	223 (18.0)
	*Y	*Y	12531 (43.2)	8262 (65.9)	No	7654 (92.6)	7077 (85.7)
Total			**29,039 (100)**	**19,621 (67.6)**		**13,860 (70.6)**	**13,024 (66.4)**

[#]Risk for developing celiac disease is any genotype carrying HLA-DQB1*02 and/or *0302
[a] DQA1*05:01-DQB1*02, [b] DQA1*02:01-DQB1*02, [c] DQA1*02:01-DQB1*02 or DQA1*05:01-DQB1*02
X is not DQB1*02, *03:01, *03:02, *06:02, *06:03, *06:04 but does not exclude homozygosity; Y denotes alleles other than DQB1*02 or *03:02

Subjects

Paper I

In Paper I, the result from the prospective population based cohort study of screening for celiac disease at 3 years of age is reported. 13,860 3-year old children were invited from June 2004 to August 2007 to participate in the CiPiS study (**Figure 7**) of whom 6206 children with HLA-risk alleles for celiac disease and 7654 children not carrying risk alleles (**Table 1**). Parents of 23 children reported that the child already had celiac disease and were excluded from the screening. In all, 1620/6206 (26.1%) children in the HLA-risk group and 1815/7654 (23.7%) children in the HLA-non-risk group participated at a median 3.2 years of age (range 2.6-4.8 years) (**Figure 8**).

Paper II

In Paper II, 13,024 of the 13,860 children invited in the first screening were re-invited to a follow-up screening at 9 years of age between June 2010 and August 2013 using the same study protocol as in the first screening (**Figure 7, Table 1**). Fifty-six children diagnosed with celiac disease at 3 years of age were excluded from the follow-up screening. In all, 1910/5947 (32.1%) in the HLA-risk group and 2176/7077 (30.6%) in the HLA-non-risk group accepted participation and were screened at median 9.2 years of age (range 8-12.8 years) (**Figure 8**). Parents of 82 children reported that the child already had celiac disease and those children were excluded from the screening.

Paper III

In Paper III, serum cytokine levels were assessed in 3-year old children with screening-detected celiac disease and potential celiac disease (i.e. persistently positive for tTGA and normal histology of biopsy), identified in Paper I, and compared with matched controls. Included were available samples from 34 children with persistently positive tTGA; 26 biopsy-confirmed celiac disease and 8 potential celiac disease and 68 tTGA negative controls matched for sample date, year of birth, gender and HLA genotype. Of the 26 children with screening-detected celiac disease, samples at follow-up were available for cytokine measurements in 16 children, after maintaining a gluten-free diet for 1.6 ± 0.4 (mean$\pm$SD) years.

Paper IV

In Paper IV, a case-control study on BMD, 25(OH) vitamin D3 and PTH was performed at the follow-up screening (Paper II) in 71 children 9 years of age at diagnosis of screening-detected celiac disease and 142 tTGA negative children matched for gender, HLA-genotype and birth year. An additional 30 tTGA negative children identified with celiac disease in the first screening (Paper I) and treated with a gluten-free diet for 6.9±1.1 (mean±SD) years were compared with another 60 tTGA negative children also matched for gender, HLA-genotype and birth year.

HLA-DQ genotyping

The HLA-DQ genotyping was performed by the same laboratory in the DiPiS study and the method is described elsewhere [229]. Shortly, high resolution HLA typing was carried out by PCR followed by hybridization with allele specific probes using DELFIA hybridization assay (Perkin Elmer Life Science, Boston, MA, USA). Two sets of probes were used to identify the alleles of DQB1; the first set containing *06:02/3, *06:03/4 and a control probe and the second set containing *02, *03:01 and *03:02. If positive for DQB1*02 the sample was further analysed for alleles of DQA1 with probes for *02:01 and *05. Alleles not identified as any of the alleles above were denoted X, but since no full typing was performed, in genotypes positive for only one of the HLA-DQB1 alleles the X did not exclude homozygosity.

Tissue transglutaminase autoantibody analysis

All samples were analysed for IgA-tTG and IgG-tTG in separate radioligand binding assays (RBA) as described elsewhere [5]. Briefly, human tTG was synthesized in the presence of ^{35}S-methionine (Perkin Elmer Life Sciences, Boston, MA, USA) by *in vitro* transcription and translation to form ^{35}S-tTG and the RBA was run as previously described but with some modifications [230]. In order to separate free ^{35}S-tTG from antibody-bound ^{35}S-tTG, the IgA-tTG antibody/antigen complexes were isolated with 10% goat anti-human IgA Agarose (Sigma, St. Louis, MO, USA) and the IgG-tTG antibody/antigen complexes were separated with 30% protein A Sepharose conjugate 4B (Zymed Laboratories, San Francisco, CA, USA). Radioactivity was measured in a beta counter and the

relative amount of tTGA was expressed as units per millilitre (U/mL) computed from standard curves.

Cut-off levels for a positive result was adapted from a previous study and set at 16 U/mL for IgA-tTGA and 4 U/mL for IgG-tTGA representing the 95th percentile of 398 healthy blood donors calculated from quantile-quantile (QQ) plots [5]. Borderline zones between a weakly and moderately positive value were arbitrarily set between 16-26 U/mL for IgA-tTGA and at 4-6.5 U/mL for IgG-tTGA, respectively. Children with a positive result in the screening were considered for a follow-up sample after 3 months and those with at least one value above borderline zones of 26 U/mL for IgA-tTGA and 6.5 U/mL for IgG-tTGA in any of the two consecutive positive samples were defined as persistently tTGA positive and qualified for intestinal biopsy.

Intestinal biopsy

In the first screening at 3 years of age, one or several biopsies were taken from the distal part of duodenum by Watson capsule or with upper endoscope performed at the local paediatric clinic to which the child was referred. In the follow-up screening at 9 years of age, all children were investigated with gastroscopy performed by a paediatric gastroenterologist at the Department of Pediatrics, at Skåne University Hospital in Malmö, Sweden, with 6 biopsies taken from the duodenum and 2 separate from bulb. Standard sections were stained with haematoxylin and eosin and biopsies from all children were stained using immunohistochemistry for CD3+ IEL. Samples were examined by a pathologist at the Department of Pathology at the same hospital and in the screening at 3 years, one pathologist re-examined all biopsies. Biopsies were classified according to the Marsh-Oberhuber classification [112, 113] with some slight modifications [231]: normal villous and crypt architecture and IEL < 25/100 enterocytes (Marsh 0); normal villous and crypt architecture but increased number of IEL (Marsh 1); normal villi but crypt hyperplasia and increased number of IEL (Marsh 2); crypt hyperplasia, increased number of IEL and partial villous atrophy (villous length> villous width) (Marsh 3A); crypt hyperplasia, increased number of IEL and subtotal villous atrophy (villous length<villous width) (Marsh 3B); crypt hyperplasia, increased number of IEL and total villous atrophy (Marsh 3C).

Celiac disease diagnosis

For the purpose of the CiPiS study, diagnosis of celiac disease was defined as persistently tTGA positivity and a Marsh score $\geq$ 1. Children being negative for tTGA in the screening were considered not having celiac disease and were included as controls in Paper III and IV. In Paper IV, adherence to a gluten-free diet was defined as children stating gluten-free diet for more than 1 year in combination with tTGA levels below the cut-off level for a positive result.

Cytokine measurements

All plasma samples in the CiPiS study were immediately stored in -20ºC after arrival. After analysis of tTGA they were once again frozen and stored. After 5.7 ± 0.9 (mean$\pm$SD) years, samples from participants in Paper III were thawed for analysis of cytokines using an electro chemi-luminescent multiplex sandwich enzyme-linked immune-sorbent (Th1/Th2) assay (MesoScale, Gaithersburg, MD, USA). All cytokines were measured at the same time in a single sample (25 μl human serum). In the chosen assay, the following cytokines and chemokine were measured: IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, TNF-α, and IL-8, as per the manufacturer's protocol on a SECTOR 6000 instrument (http://www.mesoscale.com). Blood samples taken after starting a gluten- free diet were analysed 6.1 ± 0.7 (mean$\pm$SD) years after being collected.

Dual X-ray absorptiometry

In participants in Paper IV BMC (g), BMD (g/cm^2), lean body mass, and body fat mass were measured using DXA. The first 24 children investigated were analysed using Lunar Prodigy DXA (GE Lunar Corp., Madison, WI, USA) and the following 279 children were measured utilizing Lunar iDXA (software version 13.60)(GE Healthcare, Madison, WI, USA). Cross-calibration measurements were made and the differences between measurements of the two apparatuses were considered negligible. BMC and BMD were measured in total body by a total body scan and in spine (L1-L4) by a lumbar spine scan as well as in femoral neck by a hip scan using standard software. Lean body mass and body fat mass were calculated from the total body scan.

Anthropometrics and questionnaire

In Paper IV, height (cm) and weight (kg) were measured by the same standard equipment at the DXA laboratory, and body mass index (BMI) (kg/m^2) was calculated. A questionnaire was filled out by the parents together with their child regarding lifestyle factors such as diet, physical activity, diseases, and medication.

25(OH) vitamin D3 and parathyroid hormone

In Paper IV, serum from all participants were analysed for levels of 25(OH) vitamin D3 (nmol/L) using liquid-chromatography-mass-spectrometry (Model API 4000 LC/MS/MS System) (Ab Sciex, Framingham, MA, USA). Plasma samples were analysed for PTH (pmol/L) using an Electro Chemi Luminiscence Immunoassay (Cobas 8000 (E601)) (Roche, Basel, Switzerland). All analyses were performed at the Department of Laboratory Medicine at Skåne University Hospital, Malmö. Both vitamin D and PTH levels were adjusted for season of sampling by classifying the sample date as winter (October-March) or summer (April-September) [232, 233].

Statistical methods

Statistical calculations were performed using SPSS version 14.0, 21.0 and 22.0 (www.ibm.com/software/se/analytics/spss/). Figure 1 in Paper III was drawn using GraphPad Prism version 4 (www.graphpad.com/scientific-software/prism/). In all papers descriptive data are presented as numbers (n) and proportions (%) and result data of levels of tTGA are presented as median and range (lowest-highest). The 95% confidence intervals (95%CI) were calculated for population proportions using the asymptotic method. All p-values are two-sided and a p-value < 0.05 was considered to indicate statistical significance.

In Paper I, difference in celiac disease prevalence in HLA-risk group and HLA-non-risk group was tested using Fisher's exact test with the Monte Carlo approximation. Multiple logistic regression analysis was used to calculate odds ratio of participation in relation to known factors of the HLA-genotyped population.

The statistical analysis in Paper I was done by Kristian Lynch.

In Paper II, the Fisher's exact test examined for differences in tTGA positivity and celiac disease frequencies between HLA-risk group and HLA-non-risk group. The Pearson χ^2-test examined for differences in tTGA positivity and celiac disease frequencies within the HLA-risk group between children that participated in the screening at three years and children only participating at nine years. The Mann-Whitney U-test examined differences in tTGA levels between children with celiac disease and children having normal biopsy. Correlations between tTGA levels and Marsh scores were assessed by the Spearman coefficient.

In Paper III, the Kruskal Wallis one-way analysis of variance test tested for overall differences in cytokine levels between several groups and the Mann-Whitney U-test tested for differences between the two groups of celiac disease children and controls. The difference between cytokine levels in children with celiac disease before and after a gluten-free diet was tested using the Wilcoxon´s sign-rank test. Multiple comparisons were corrected for using the Bonferroni correction method.

In Paper IV, differences in age at tTGA measurement and DXA examination were calculated using Student´s t test. Differences between cases and controls in life style factors were tested using Pearson Chi Square test or Fisher's exact test if small sample sizes. Group differences in anthropometrics, BMC, BMD, vitamin D, and PTH were measured using ANCOVA to adjust for covariates. Correlations between BMD and tTGA, Marsh scores, levels of vitamin D, and PTH were assessed by the Spearman coefficient.

Ethical approvals

The Ethics committee at Lund University approved the CiPiS study regarding screening at 3 years of age (Paper I) in 2004 (Dnr LU 878-02). In the follow-up study at 9 years of age including DXA measurements (Paper II, IV) the research project was approved in 2010 by the Regional Ethics Review Board in southern Sweden (Dnr 2010/170). The Radiation Safety Committee at Skåne University Hospital, Malmö approved the DXA measurements in June 2010. An additional application concerning cytokine measurements (Paper III) was approved by the Regional Ethics Review Board in southern Sweden in 2011 (Dnr 2011/181). Parents or caregivers of participating children gave their written consent to participate in the screening for celiac disease including, if offered, DXA measurements.

Results and discussion

Paper I

Screening detects a high proportion of celiac disease in young HLA-genotyped children

Celiac disease is considered to be a common and often subclinical disease where the majority can only be identified in screenings with tTGA [140]. It is considered to be found only in individuals with genetic susceptibility but few studies have been conducted among both children at HLA-risk and children at HLA-non-risk [75]. We therefore set out to investigate the prevalence of screening-detected celiac disease using detection of tTGA in a cohort of 3-year old children born in Skåne June 2001-August 2004 which had become HLA-genotyped at birth thus consisting of children with both HLA-risk and HLA-non-risk.

Persistently positive tTGA was found in 73/1620 (4.5%, 95% CI=3.5-5.5%) children with HLA-risk, but none among the 1815 children in the HLA-non-risk group (p<0.001) (**Figure 8**). Among the persistently tTGA positive, 59/73 (81%) carried HLA-DQB1*02, 6/73 (8%) HLA-DQB1*03:02 and 8/73 (11%) carried both alleles.

Seventy-one of the 73 tTGA positive children underwent intestinal biopsy of whom 56 were diagnosed with celiac disease. Six of these children had an intestinal biopsy classified as Marsh 1 and 50 children Marsh 3. The remaining 15 (21%) children had normal biopsies and were classified as having potential celiac disease. In all, 56/1618 (3.5%, 95% CI=2.6-4.4%) children were diagnosed with celiac disease (**Figure 8**).

Children born in the province of Skåne in Sweden between June 2001 and Aug 2004 (N≈39,000)

HLA genotyped newborns N=29,039

HLA-DQ2 and/or DQ8   HLA-non-DQ2 and non-DQ8

Invited to 1st screening at age 3 years between June 2004 and Aug 2007 (N=13,860)

Participated at 3 years N=1620   Participated at 3 years N=1815

Outcome of screening at 3 years of age

tTGA+* N=73 (4.5%)   tTGA- N=1547   tTGA+* N=0   tTGA- N=1815

Diagnosis of biopsy-proven celiac disease?

Yes N=56 (3.5%)   No

Invited to 2nd screening at age 9 years between June 2010 and Aug 2013 (N=13,024)

Enrolled at 9 years N=930   Rescreened at 9 years N=980   Rescreened at 9 years N=1078   Enrolled at 9 years N=1089

Outcome of screening at 9 years of age

tTGA+* N=46 (4.9%)   tTGA+* N=33 (3.4%)   tTGA+* N=0   tTGA+* N=1#

Diagnosis of biopsy-proven celiac disease?

Yes N=42 (4.5%)   Yes N=30 (3.1%)   No   No

*Persistence of IgA-tTG and/or IgG-tTG in two samples 3 months apart; #IgG-tTG positive only.

Figure 8. Participation and outcome of screening at 3 years of age (solid line) respective 9 years of age (dotted line for children already screened at 3 years and dashed line for children screened for the first time at 9 years) according to HLA.

The finding that all children with tTGA were found in the HLA-risk group indicates that screening could be restricted to children at genetic risk. On a population level this suggests that population-based prospective screening for celiac disease could be performed in a selected group of children using HLA-genotyping as a first step and tTGA as a secondary step [130]. In our HLA-genotyped cohort, 56.8% carried these risk alleles further excluding nearly half of the population in a screening. It has been argued that HLA-genotyping would induce unnecessary anxiety and risk of misinterpretation [234]. However, most parents of children participating in screenings do not consider the result of HLA-genotyping a health disadvantage [235]. A second approach is using HLA-genotyping as a confirmatory step in tTGA positive children, which has been applied in many previous screening studies [73, 236]. However, this approach would not have added any useful information in predicting celiac disease in our study.

The high prevalence (3.5%) of screening-detected celiac disease in HLA-risk children is comparable with other population-based studies following children at HLA-risk (1.2-4.8%) [22, 90, 91]. It also supports other studies indicating that Sweden is a country with high prevalence of undetected celiac disease [237, 238].

Still, 21% of children with repeatedly positive tTGA undergoing intestinal biopsy had normal histological findings yielding a positive predictive value of only 79%. It has been argued that tTGA positive children having a normal histology are falsely positive, but tTGA can fluctuate over time in genetically susceptible individuals that could not be explained simply by laboratory factors [91]. We consider the children with persistent tTGA to be true tTGA positives since they were repeatedly positive in samples taken approximately 3 months apart [239] and also because the study protocol included a higher cut-off for intestinal biopsy [240] and they were therefore classified as potential celiac disease patients. A drawback of the study was that some of the tTGA positive children were investigated with only one biopsy using a Watson capsule. It cannot be ruled out that some of these children had patchy lesions and therefore were misdiagnosed [241].

Another limitation of the study was the sample selection from the population and the low participation rate which both confer risk of sampling bias. First, the children selectable for inclusion in the study needed to be HLA-genotyped at birth in the DiPiS study, which was true for approximately 70% of the studied cohort and no information was obtained from the remaining children. Second, only children of parents accepting participation in the DiPiS study were eligible for invitation comprising of 19621/29039 (68%) of the HLA-genotyped population. At both levels of selection, since the DiPiS study is a study of T1D risk factors, families having a member affected by T1D could be more prone to participate and since relatives to T1D patients have an increased risk of other autoimmune diseases, such as celiac disease, this could cause ascertainment bias [242].

In order to identify any sampling bias, we calculated odds ratio for participating in CiPiS screening, based on information in the registration forms (see paragraph about DiPiS study), and found that, in the HLA-risk group, being a girl (OR 1.1, 95% CI=1.0-1.3; p=0.05), having a mother with diabetes (OR 3.3, 95% CI=2.3-4.9; p<0.001), having a mother with gestational diabetes (OR 1.8, 95% CI=1.4-2.4; p<0.001), or having an older mother >34 years (OR 2.2, 95% CI=1.7-2.8; p<0.001) were factors associated with participation in the CiPiS study in the HLA-genotyped population. The reason for T1D mothers and older mothers being more prone to let their children participate, was probably due to the inclusion criteria for the follow-up in the DiPiS study and these DiPiS participants were in turn offered to participate in the CiPiS study. Being a girl and having a mother with T1D are known risk factors for the development of celiac disease [243, 244] and these

factors may have led to an increase in the number of detected cases. In contrast, in the HLA-non-risk group, girls were less prone to participate (OR 0.9, 95% CI=0.9-1.0; p= 0.02) whereas prematurity (OR 1.3, 95% CI=1.1-1.4; p= 0.02) and older mothers >34 years (OR 3.4, 95% CI=2.6-4.5; p<0.001) were associated to participation in screening, which are risk factors not associated to celiac disease [244]. Overall, non-genetic factors most likely played a minor role compared to the effect of alleles of the HLA region.

It is well known that DQ2 homozygous and DQ2-DQ8 heterozygous children are at the highest risk for celiac disease and T1D respectively [22, 26]. The study design of inviting children in the HLA-risk group from children already participating in the DiPiS study led to that these HLA-DQB1 genotypes were selected for invitation to participation to a higher extent thus resulting in risk for sampling bias (**Table 1**). Having a family member with celiac disease or symptoms associated to celiac disease could also have affected the propensity for participating, which was not accounted for as an additional risk for sampling bias. Accordingly, the estimated prevalence cannot be considered to represent the true prevalence of celiac disease in the study population without a risk for an upward inflation of the number of children detected with celiac disease.

In our study, persistently tTGA positive children with Marsh 1 lesions, constituting of 11%, were defined as having celiac disease. Since this definition is not supported by the present diagnostic criteria [82], the true prevalence of celiac disease may have been overestimated but still, the prevalence would reach approximately 3% if these children were excluded. Other causes linked to intraepithelial lymphocytosis should be considered (e.g. cow´s milk hypersensitivity, giardiasis, Crohn´s disease) [245] and additional diagnostic measurements have been proposed [246, 247] but these methods were not used in this study. However, the diagnostic criteria not considering children with Marsh 1 as celiac disease cases have been questioned since both children and adults with tTGA and mild intestinal inflammation also seem to benefit from a gluten-free diet [147-149, 248].

In conclusion, we found screening-detected celiac disease in 3.5% of children carrying HLA-DQB1*02 and/or *03:02 but in none of children not carrying any of these HLA-risk alleles. If mass screening is considered, our results imply that HLA-genotyping could be used to select large populations to be screened for celiac disease.

Paper II

Repeated screening is necessary for detection of celiac disease but can be restricted to at genetic risk birth cohorts

Since celiac disease may affect individuals at any time throughout life, we next decided to repeat the screening procedure in the same cohort of children as at 3 years of age in a follow-up screening at 9 years of age.

In the HLA-risk group, 79/1910 (4.1%, 95% CI=3.2-5.0) children were persistently tTGA positive compared to only 1/2167 (0.05%) in the HLA-non-risk group (p<0.001) (**Figure 8**). In the HLA-risk group, 45/79 (57%) carried HLA-DQB1*02, 18/79 (23%) carried HLA-DQB*03:02 and 16/79 (20%) carried both alleles. The tTGA positive child in the HLA-non-risk group was positive for both HLA-DQB1*06:02/3 and *06:03/4 (i.e. either 06:02/06:03, 06:03/06:04, 06:03/X or 06:02/06:04), but the HLA-DQA1 genotype was not examined and therefore it cannot be ruled out that this child had HLA-DQA1*05; an unusual risk allele for celiac disease [15]. This particular child had repeatedly elevated levels of IgG-tTG at mean 13.4 U/mL but was negative for IgA-tTG and biopsy showed normal histological features thus excluding celiac disease.

Intestinal biopsy was performed in 77 children of whom 72 were diagnosed with celiac disease (7 had Marsh 1 and 65 had Marsh 3A or higher). Five of the 77 (6%) children had normal intestinal mucosal features and were therefore classified as having potential celiac disease. In all, we found celiac disease in 72/1907 (3.8%, 95% CI=2.9-4.6) children at HLA-risk. In the HLA-risk group, 980/1910 (51%) children had previously been screened at 3 years of age. Among those, an additional 30/979 (3.1%, 95% CI=2.0-4.1) children were diagnosed with celiac disease compared with 42/928 (4.5%, 95% CI=3.2-5.9) new patients of those that participated for the first time at 9 years of age (p=0.09) (**Figure 8**).The Kaplan-Meier estimate of cumulative incidence of celiac disease at 9 years of age was 6.5%.

Seven out of the 15 children with potential celiac disease at 3 years of age also participated in the follow-up screening at 9 years of age of whom four (4) had seroconverted to tTGA negative, two (2) developed celiac disease and one (1) remained as having potential celiac disease.

The incidence rate (IR) of screening-detected celiac disease at 3 years of age was 11.0/1000 person years (covering from 0-3 years) compared to 5.0/1000 person years at 9 years of age (covering from 3-9 years) (IR ratio=0.4, 95%CI=0.3-0.7). In children carrying DQ2/X, i.e. being HLA-DQA1*05:01-DQB1*02 homozygous or heterozygous in combination with other alleles, IR was 27.4/1000 person years

at 3 years of age and 7.9/1000 person years at 9 years of age (IR ratio=0.3, 95%CI= 0.1-0.6).

Accordingly, all children with biopsy-confirmed celiac disease were found in the HLA-risk group thus confirming the results of the screening at 3 years of age and suggesting that the screening procedure of serial testing with tTGA could preferably be preceded by HLA genotyping. The finding of an additional 30 cases of celiac disease in the group of children that had already been screened for celiac disease clearly demonstrates the need for repeated screening during childhood and underlines the screening criteria of WHO stating that screening should be a continuing process and not a "once and for all" project [128].

To compare celiac disease frequencies in different HLA-genotypes at 3 years of age and at 9 years of age, we calculated the incidence of celiac disease and stratified it according to HLA-DQB1 genotype. The overall incidence was lower between 3-9 years compared to 0-3 years (IR ratio=0.4, 95%CI=0.3-0.7) and this was also true in children carrying DQ2/X (IR ratio=0.3, 95%CI= 0.1-0.6). The incidence of screening-detected celiac disease seems to be higher in young preschool children compared to older pre- and schoolchildren and particularly in children carrying DQ2/X including DQ2 homozygous individuals. The finding in DQ2/X children is supported by a former screening study in which DQ2 homozygous children developed celiac disease at an earlier age [22] although this was not found in studies of clinically detected celiac disease [249, 250]. On the contrary, this pattern was not true for any of the other genotypes and the overall result did not support a stratification of screening time-point according to HLA-genotype.

Further, only half of the children with potential celiac disease from the screening at 3 years of age participated in the screening at 9 years of age. Despite this fact, the follow-up screening of these children indicated that the majority (57%) with potential celiac disease at a young age had seroconverted to tTGA negative at an older age. This supports the belief that a substantial proportion of tTGA positive children having normal mucosa do not develop celiac disease at follow-up [125, 146]. The proportion of potential celiac disease among the children undergoing intestinal biopsy was 5/77 (6%) in children at 9 years of age compared to 15/71 (21%) in children at 3 years of age (p=0.01). One explanation could be different use of diagnostic procedures between the first and second screening where the latter applied multiple biopsies through upper endoscopy in all children. Another reason for the difference in the percentage could in fact be that younger children are more prone to have potential celiac disease and that the diagnostic performance of the tTGA test may vary in different age-groups. In addition, levels of tTGA in potential celiac disease children were significantly lower than in children having celiac disease and it has previously been demonstrated that tTGA

levels correlate with Marsh score [99, 251]. A future screening procedure could consider to adjust tTGA cut-offs for intestinal biopsy among different age-groups or to add an additional antibody test such as EMA in those children with low levels in order to increase the positive predictive value and lower the number of unnecessary biopsies [234].

The study design, as discussed in Paper I, regarding different invitation frequencies in certain HLA DQB1 genotypes probably affected the outcome also in this study (**Table 1**). Additional analysis of factors related to participation was not made in Paper II based on the assumption that the known population characteristics from the registration form filled in at birth mostly related to perinatal conditions and considered of minor importance at nine years of age. The low participation rate may also have increased the effect of sampling bias and thereof the detection rate of affected cases due to risk for greater participation of individuals affected by celiac disease related health problems or having a first degree relative with celiac disease [252]. Supposing all cases were identified in the invited population with HLA-risk, this would reduce the prevalence to 72/5947 (1.2%). Before a mass screening procedure is considered, factors associated with participation and non-participation needs to be further investigated.

In conclusion, repeated screening is necessary to identify new patients by 9 years of age but screening may be restricted to children at genetic risk.

Paper III

Serum cytokine pattern in young children with screening detected coeliac disease

Previous studies of subclinical manifestations in celiac disease have been performed in individuals with clinically detected celiac disease or in celiac disease detected by screening in individuals from risk groups such as T1D [253]. These results cannot automatically be applied to children with screening-detected celiac disease from the general population and any possible benefit from identification and treatment of these children needs to be evaluated.

Systemic cytokines are suggested to be potential links between celiac disease and complications such as bone loss [217, 218]. In order to examine signs of systemic inflammation, we measured 10 different cytokines in serum of 3-year old children with screening-detected celiac disease (identified in Paper I) at time of diagnosis and after treatment with a gluten-free diet.

Cytokine measurements in serum from 26 children with screening-detected celiac disease were compared with 52 tTGA negative matched controls and showed a significant increase in cases regarding the cytokines IFN-γ, IL-5, IL-10, IL-12p70, and IL-13 whereas no difference was found between children with celiac disease and controls for the cytokines TNF-α, IL-1β, IL-2, IL-4, and IL-8. The result remained unchanged if analysing subgroups of children having Marsh score 1 (n=3) and 3 (n=23) respectively. There was no difference between the 8 children having tTGA and normal intestinal biopsy (i.e. potential celiac disease) and controls in any of the systemically measured cytokines.

Blood samples were collected in 19 of the 26 children with screening-detected celiac disease after being on a gluten-free diet for 1.6 ± 0.4 (mean$\pm$SD) years. Levels of IL-5, IL-10 and IL-12p70 showed a significant reduction in cytokine levels after starting a gluten-free diet and IFN-γ levels were reduced although not significantly. No difference was seen in IL-13 after starting a gluten-free diet.

The finding of elevated cytokines detected in serum is in line with findings in clinically detected celiac disease [65]. The cytokine pattern in celiac disease has been mainly attributed to cytokines of the T_H1 response [67] which are cytokines, dominated by IFN-γ, exerting their effect through facilitating cell-mediated responses. This was confirmed in our study showing elevated levels of IFN-γ and IL-12p70. We also found elevated levels of IL-5, IL-10, and IL-13 traditionally associated to the T_H2 pattern, which also have been found in a former study of celiac disease appealing to the combined pattern of T_H0 in celiac disease [65]. The increase in IL-10, an anti-inflammatory cytokine and important tool of intestinal regulatory T cells for exerting gut tolerance, probably reflects a balancing anti-inflammatory response and this cytokine have been found to be elevated in previous studies [66, 254]. The increase of several cytokines in serum suggests that children with celiac disease detected by screening have signs of systemic inflammation reflected as an increase in pro-inflammatory cytokines.

IL-5, IL-10, IL-12p70, and IFN-γ were down-regulated after starting a gluten-free diet. Reduction in cytokine levels as a response to a gluten-free diet has been previously demonstrated in clinically detected celiac disease [69, 71]. Interestingly, children with potential celiac disease did not differ from the controls in measured cytokines and both these findings suggest systemic inflammation to be dependent of mucosal inflammation maybe reflecting a dose-dependent relationship.

Several cytokines have been linked to complications associated with celiac disease such as B-cell lymphoma [255], T-cell lymphoma [256], anaemia [257], and osteoporosis [217, 218]. Our finding, that pro-inflammatory cytokines are elevated in serum of children with screening-detected celiac disease and down-regulated during a gluten-free diet, supports the hypothesis that these cytokines could serve

as a link between untreated celiac disease and related complications and that a gluten-free diet decreases the risk of these long-term complications.

A draw-back of the study was the long and varying storage time between sample collection and analysis (see paragraph about cytokine measurements). Analysis of cytokine levels are thought to be affected by storage conditions, storage time and number of thaw cycles [258, 259]. However, all measurements were performed on samples that had been stored and frozen in the same manner and thawed for the purposes of this study only. Since samples of controls were matched for sample date in cases, there was no difference in storage time between cases and controls.

In conclusion, signs of systemic inflammation, reflected as elevated levels of serum cytokines, were found in 3-year old children with screening-detected celiac disease at time of diagnosis. A chronic systemic inflammation due to unrecognized disease in young children could potentially contribute to an increased risk for complications associated with untreated celiac disease. This finding, if confirmed in additional future studies, should be accounted for when considering screening in young children from the general population.

Paper IV

Bone mineral density in children with screening-detected celiac disease: a case-control study

A well-known subclinical complication of celiac disease is low BMD found in both children and adults [192, 260, 261]. Celiac disease is also associated with an increased risk for fractures [209]. However, it is not known if low BMD is a complication found also in children with celiac disease detected by screening. If so, this could serve as an additional argument for celiac disease screening in children from the general population. In Paper IV, we set out to measure BMD, 25(OH) vitamin D3 and parathyroid hormone (PTH), at diagnosis, in children with screening-detected celiac disease at 9 years of age and in children with the same diagnosis but on a gluten-free diet for 6.9 ± 1.1 (mean$\pm$SD) years. We designed it as a nested case-control study within the CiPiS study described in Paper II.

At diagnosis, children with screening-detected celiac disease, compared to controls, had lower BMD of total body (mean 0.84 g/cm^2 (95%CI=0.82 to 0.86) vs. mean 0.87 g/cm^2 (95%CI=0.86 to 0.88); p=0.009) and spine (mean 0.76 g/cm^2 (95%CI=0.74 to 0.77) vs. mean 0.79 g/cm^2 (95%CI=0.78 to 0.80); p=0.005).

Consequently, also children with celiac disease identified by screening seem to have low BMD as demonstrated in clinically detected celiac disease indicating that

these two phenotypes share common features. Similarly, 25(OH) vitamin D3 levels were lower in screening-detected children compared with controls (mean 65.7 nmol/L (95% CI=60.9-70.4) vs. mean 77.1 nmol/L (95%CI=73.8-80.3); p<0.001) and PTH was higher (mean 4.2 pmol/L (95% CI=3.9-4.5) vs. mean 3.2 pmol/L (95% CI 3.0-3.4); p<0.001). There was a weak correlation between D vitamin levels and BMD of total body (r=0.28; p=0.03).

Lower vitamin D levels have been found in clinically detected disease [262] although most studies do not report this finding [263-265]. One reason for lower vitamin D levels in celiac disease individuals is a reduced intake of dairy products due to lactose intolerance, but in our study consumption of dairy products did not differ between cases and controls. Another reason is malabsorption, which could be a plausible explanation in the children with screening-detected celiac disease since the majority had villous atrophy. In addition, exposure to sun light was not controlled for in our study, except for adjusting calculations to season of sample date, and therefore it cannot be ruled out that this could have affected the outcome. Nevertheless, lower vitamin D levels could have a negative effect on bone parameters in children with screening-detected celiac disease. In line with this result was the finding of higher levels of PTH in screening-detected cases compared to controls. Increased levels of PTH, as a result of a hypocalcaemic state due to malabsorption, could be one reflection of increased bone resorption leading to lower BMD and secondary hyperparathyroidism has also been found in clinically detected celiac disease [193, 197, 262]. Altogether, these findings suggest that children with celiac disease detected by screening have the same subclinical finding of low BMD and associated metabolic alterations as children with clinically detected disease, already at 10 years of age, further arguing for an early identification of the disease.

In Paper IV, children who were diagnosed with screening-detected celiac disease at 3 years of age and maintaining a gluten-free diet were compared with matched controls. Interestingly, there was no difference in BMD, 25(OH) vitamin D3 and PTH levels between children on a gluten-free diet and controls. A limitation of the study was that we did not measure these children before the initiation of the diet and therefore we cannot estimate any effect of the treatment. A gluten-free diet in children with clinically detected celiac disease have been shown to improve BMD at follow-up and studies have found complete normalization of BMD within a year [192, 266] as well as incomplete recovery after 1-2 years [197, 198]. The examined children in our study were young at diagnosis, median age being 3.2 years, and they had been consuming a gluten-free diet for almost 7 years at the time of DXA examination. Nevertheless, the results indicate that the identification of screening-detected celiac disease at a young age, with the initiation of a gluten-free diet, probably protects from acquiring low BMD at school age.

Paper IV also revealed that there was no difference between children with celiac disease detected by screening and controls regarding anthropometric measurements such as weight (p=0.23), height (p=0.43), BMI (p=0.22), lean mass of total body (p=0.15) and fat mass of total body (p=0.39). This finding suggests that pronounced malnutrition is not a major feature of children with screening-detected celiac disease and it also indicates that anthropometric tools are not reliable when it comes to identifying screening-detected celiac disease [99].

The study may be criticized for not staging pubertal maturation and thereof we could not adjust for differences in skeletal maturation, which is more closely associated to mineral accrual than chronological age [267]. Timing of puberty affects bone mineral acquisition and adolescents with a later onset of maturation may have lower peak bone mass [268]. Delayed puberty is considered a common extra-intestinal symptom of celiac disease and it cannot be ruled out that this condition affected the results of the BMD measurements.

In conclusion, children with celiac disease identified by screening have lower BMD, lower vitamin D3, and higher PTH compared with matched controls. These differences are not found in celiac disease children on a gluten-free diet diagnosed at an earlier age. These findings suggest that children with screening-detected celiac disease could benefit from early identification and treatment.

Preliminary findings

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Conclusions

- Screening-detected celiac disease is only found among children at genetic risk for the disease.

- Screening for celiac disease needs to be repeated during childhood in order to identify new patients.

- HLA-genotyping could be used to select large populations to be screened for celiac disease.

- Children with screening-detected celiac disease show signs of systemic inflammation reflected as elevated pro-inflammatory cytokines in peripheral blood which decline after starting a gluten-free diet.

- Children with screening-detected celiac disease have affected bone mineral density at time of diagnosis which is not found in children on a gluten-free diet.

- Children with screening-detected celiac disease may therefore benefit from early identification and treatment.

Future perspectives

The knowledge about celiac disease being a common disease affecting many children and adults of the general population has evolved the last 25 years in the light of several larger prevalence studies [75, 77, 80]. Studies have revealed that the prevalence of celiac disease is high in the Swedish population as compared to other countries [22, 237, 238]. The increased awareness that celiac disease does not typically present itself with classical clinical symptoms [92, 97] has led to the conclusion that the identification of celiac disease patients requires a proactive and broad approach.

The search for celiac disease by active case finding, i.e. screening all individuals seeking medical attention for symptoms that could be attributed to celiac disease in any way and screening risk groups, has been proven effective in increasing prevalence of celiac disease [269, 270]. Still, there are several prerequisites for this approach. First, it relies on the individuals´ ability to seek medical attention for even subtle symptoms, which in turn evokes the need for health education of the general population. Second, there is a substantial need for education of the personnel of these medical facilities in the myriad of symptoms that can be linked to celiac disease in different age groups and all potential risk groups such as second degree relatives [271]. Even if so, there is still evidence that some individuals with celiac disease do not have symptoms that lead them to medical attention or are truly asymptomatic and these individuals would not be identified by active case finding [99, 272, 273]. The complex presentation of celiac disease is also the reason why I have applied the expression "screening-detected celiac disease". As it implies, it includes all individuals that have not yet been identified by seeking medical attention, regardless of the clinical picture. Symptoms are subjective measurements, especially in preschool children where parents are the interpreter of their child´s behaviour, and are uncertain test methods for identification of celiac disease even in prospective studies [99, 100]. Active case-finding also include screening for celiac disease in at-risk groups which is part of the current diagnostic recommendation from ESPGHAN [82]. This has led to an increased knowledge about celiac disease among these groups [38, 274, 275] and studies have shown health-benefits from screening but also questioned if there are any advantages [253].

Since celiac disease is a common disease of Western populations and often remain undetected, a third way of searching for the disease would be by mass screening in the general population. This method is being discussed, but still, not all WHO criteria are fulfilled to institute national or international screening programs [132]. The results from the CiPiS study clearly contribute to the fulfilment of these criteria in several ways. First, the finding of screening-detected celiac disease in more than 3% of a cohort of children at HLA-risk. Second, the finding that none of the celiac disease children identified through repeated screening were found among children not at genetic risk. These findings clearly indicate that, if mass screening should be performed using tTGA, this test needs only to be repeatedly performed in children having the alleles of HLA-DQB1*02 and/or *0302.

However, there are several aspects of HLA-genotyping that have to be considered. First, there are ethical aspects of identifying children at genetic risk who potentially can develop a disease although the absolute risk is low [276]. The cost has been used as an argument against the procedure but the last ten years simple non-costly SNP tests have been developed [277]. There are insurance policy issues that can affect the individual in a negative way [278]. If the identified alleles could be coupled to other unrelated health aspects in the future, the results from the HLA-genotyping could potentially be used in an unwanted way. These arguments against HLA-genotyping shall be contrasted to the ethical aspects of repeatedly screening children and adults not having these HLA-risk alleles who will, most likely, never develop celiac disease. Moreover, the HLA-genotyping is accepted in the medical association since the algorithm for screening children in at risk groups contains HLA-DQ genotyping as the first step [82]. The general attitude towards HLA-genotyping among celiac disease families seems also to be supportive [235], but the view of the parents to children of the general population regarding HLA genotyping that estimates celiac disease risk is not known.

The results from the CiPiS study also highlight the aspect of how to maximize participation in screening, especially since the results indicate that the screening needs to be repeated in formerly unaffected children. The study design in combination with the low participation rate led to difficulties in applying the result of the cohort screening to the general population. Despite repeated letters of reminder, phone-calls from study nurse, and the possibility to obtain the blood sample at home and to send it by mail to the research centre the participation rate was < 30%. Factors affecting participation and non-participation as well as general attitudes towards celiac disease screening needs to be evaluated and experience from already established screening programs needs to be obtained.

Among the WHO criteria needed to be fulfilled if screening of the general population is to be considered, is the first criteria; the condition sought should be an important health problem [128]. This is clearly true for symptomatic

individuals but not fully understood in asymptomatic cases [132]. The basis for this is the view that celiac disease probably consists of different subtypes of the disease, perhaps holding different risks of complications if untreated. The first effect of a gluten-free diet is of course symptom-relief, which will not be fulfilled in truly asymptomatic cases. The second effect could be in subclinical measurements such as nutritional parameters, which of course could not be self-evaluated by the individual but instead needing medical evaluation. The CiPiS study demonstrates that children with screening-detected celiac disease have subclinical signs of disease consisting of systemically increased levels of pro-inflammatory cytokines in peripheral blood and low bone mineral density which is not found in patients on a gluten-free diet. We have not evaluated the hard endpoints of these findings, i.e. if the systemic inflammation could be coupled to an organ-specific complication or if the low BMD would lead to increased risk of future fractures, which would need a longitudinal follow-up study. However, in Paper IV we did not find any difference in fracture frequency between cases and controls although this was only evaluated through questionnaires and a more validated method of fracture frequency determination, such as a register-study or medical record-study, could be applied. Also, an assessment of treatment effect could be evaluated in a future longitudinal follow-up instead of the presented cross-sectional method. The effect of a gluten-free diet on BMD and fractures could preferentially be assessed in a randomized controlled study, which would also evaluate the natural history of screening-detected celiac disease.

Nevertheless, the presented results from the CiPiS study are the first step in estimating subclinical properties of screening-detected celiac disease in children. In the future, the cohort of children with screening-detected celiac disease could be used in additional testing of subclinical signs of celiac disease, e.g. retrospective and prospective study of dental records that could reveal if these children have increased prevalence of dental enamel defects [279]. The cost-effectiveness of screening needs extensive future research and maybe the CiPiS cohort could be used as a screening model in such calculations.

Populärvetenskaplig sammanfattning

Celiaki, även kallat glutenintolerans, är en tarminflammation orsakad av vissa sädesslag i kosten och förekommer hos både barn och vuxna. Sjukdomen drabbar ca 1% av befolkningen i västvärlden och betraktas som en av de vanligaste kroniska sjukdomarna bland svenska barn. Många individer med sjukdomen är oupptäckta och kan bara hittas via s.k. screening, d.v.s. att man letar efter sjukdomen hos tillsynes friska individer. I studierna, som ligger till grund för den här avhandlingen, visar vi att screeningupptäckt celiaki enbart drabbar barn med genetisk risk och att dessa barn uppvisar tecken på inflammation i blodet samt har nedsatt bentäthet vilket barn med behandlad celiaki inte har. Dessa fynd talar således för att det finns en fördel med att tidigt hitta och behandla dessa barn och att man bör överväga screening av alla svenska barn med genetisk risk.

Vad är celiaki?

Gluten, som orsakar celiaki, är samlingsnamnet för protein i sädesslagen vete, råg och korn. Sjukdomen innebär att immunförsvaret i tunntarmen reagerar på gluten i kosten och utlöser en inflammation i tarmen som skadar tarmluddet. Skadan på tarmluddet kan leda till att man får obehag från magen, t.ex. diarré eller magont, men också till besvär utanför tarmen, t.ex. trötthet och ledvärk. Tarmskadan kan också leda till nedsatt näringsupptag och näringsbrist som i sin tur leder till att man som barn växer sämre. Obehandlad celiaki är också förknippad med nedsatt bentäthet, s.k. benskörhet, hos både barn och vuxna. Varför man får nedsatt bentäthet är inte klarlagt men det kan bero på näringsbrist, t.ex. brist på av kalcium och D-vitamin. Det kan också bero på att inflammationen i tarmen påverkar benomsättningen i skelettet t.ex. via frisättning av s.k. cytokiner, vilket är signalmolekyler som frisätts av immunceller vid inflammation.

Hur upptäcks celiaki?

Studier av sjukdomsförekomst har förvånansvärt visat att majoriteten av individer med celiaki är oupptäckta, sannolikt p.g.a. att de inte har typiska symptom eller helt enkelt saknar besvär. Om man ska hitta alla som är drabbade av celiaki måste man därför undersöka även de som inte har uppenbara besvär via s.k. screening, en metod som innebär att man letar efter sjukdom hos tillsynes friska individer. Celiaki är starkt kopplat till förekomst av vävnadstransglutaminas-antikroppar, som kan mätas via ett blodprov. Detta test kan därför användas som metod vid screening, men för att säkert konstatera sjukdomen måste tarmen undersökas med vävnadsprovtagning. Sjukdomen är också förknippad med vissa varianter av gener i den s.k. HLA-regionen på kromosom 6. Denna region kodar för en molekyl som presenterar gluten-proteinet för immunförsvarets celler. De allra flesta individer med celiaki har en av två varianter av dessa gener. Dessa två varianter är dock vanliga i den svenska befolkningen (ca 30-40% har någon av dessa) och att ha dessa riskgener betyder inte att man drabbas av sjukdomen utan endast att man har risk att drabbas. Har man ingen av dessa varianter är risken väldigt liten att man någonsin drabbas av celiaki. Om man vid screening behöver undersöka de som inte har genetisk risk är dock oklart då de flesta tidigare screening-studier inte undersökt den genetiska risken hos alla studie-deltagare.

Hur behandlar man celiaki?

Celiaki behandlas med glutenfri kost, d.v.s. kost som inte innehåller glutenprotein. Kostbehandlingen leder för de allra flesta till att inflammationen i tarmen läker ut och att tarmluddet återställs. Då kan tarmen fungera som vanligt och de eventuella besvär som individen har haft försvinner. Efter att man börjat med glutenfri kost förbättras bentätheten hos alla med celiaki och den normaliseras hos barn. Om det finns hälsofördelar med glutenfri kost för de som inte har uppenbara besvär av sjukdomen och vars sjukdom endast kan upptäckas via screening är dock oklart då fördelar med behandlingen mest är studerat hos individer som söker sjukvård för besvär eller som har annan känd sjukdom som är kopplad till celiaki t.ex. diabetes.

Hur gick forskningsstudierna till?

Syftet med forskningen, som den här avhandlingen bygger på, var att upprepat undersöka förekomsten av screeningupptäckt celiaki hos barn med kartlagd genetisk risk respektive icke-risk och studera faktorer hos dessa barn som skulle kunna motivera till upptäckt av sjukdomen och behandling av densamma.

Under åren 2001-2004 bjöds föräldrar till alla nyfödda barn i Skåne in till forskningsstudien DiPiS (Diabetes Prediktion i Skåne). Barn som deltog HLA-bestämdes (d.v.s. deras genetiska risk och icke-risk bestämdes) via blodprov från navelsträngen. Vid tre års ålder, under åren 2004-2007, bjöds 13 860 barn in till screeningstudien CiPiS (Celiaki Prediktion i Skåne) och de som deltog skickade in ett blodprov från barnet för analys av vävnadstransglutaminas-antikroppar. Både barn med och utan genetisk risk deltog. Om upprepade blodprov innehöll antikroppar undersöktes barnet med hjälp av tarmundersökning för att fastställa sjukdomen. De barn som hade celiaki rekommenderades glutenfri kost. Samma grupp av barn, förutom de som redan fått diagnosen celiaki vid 3 års ålder, bjöds återigen in under åren 2010-2013, nu vid nio års ålder, för upprepad screening på samma sätt som tidigare.

Vid tre års ålder analyserades också blodprover från barn med nyupptäckt celiaki avseende signalmolekyler som frisätts vid inflammation, s.k. cytokiner. Dessa analyserades vid tidpunkten för diagnos samt efter att barnen börjat med glutenfri kost och jämfördes med barn utan antikroppar, s.k. kontroller. Vid nio års ålder undersöktes barnen med nyupptäckt celiaki med bentäthetsmätning och blodprov analyserades för D vitamin och parathormon. De barn som fått celiaki vid tre års ålder och som hade glutenfri kost undersöktes på samma sätt och alla jämfördes med kontroll-barn.

Vilka är resultaten och slutsatserna?

Vid tre års ålder deltog totalt 3435 barn och 3,5% av barnen med genetisk risk hade celiaki och inget barn utan genetisk risk hade celiaki. Vid nio års ålder deltog 4077 barn och 3,8% av barnen med genetisk risk hade celiaki och inte heller här hittades något barn med celiaki bland barn utan genetisk risk. Slutsatsen av detta är att screeningupptäckt celiaki är vanligt bland svenska barn med genetisk risk och om man skall genomföra screening i befolkningen bör denna riktas enbart mot de som har genetisk risk, d.v.s. ca 30-40% av befolkningen, men måste upprepas då nya individer insjuknar under barnaåren.

De treåriga barnen med screeningupptäckt celiaki hade förhöjda nivåer av inflammatoriska cytokiner i blodet jämfört med kontroller och de flesta av dessa cytokiner minskade i blodet hos barnen efter ändring till glutenfri kost. De nioåriga barnen med samma sjukdom hade låg bentäthet, lågt D vitamin och förhöjt parathormon jämfört med kontroller. Däremot hade barnen med glutenfri kost sedan 3 års ålder samma värden som kontrollerna. Slutsatsen är att tillsynes friska barn med screeningupptäckt celiaki har tecken på inflammation och låg bentäthet med påverkade näringsvärden jämfört med friska barn, men när de har glutenfri kost är värdena samma som andra barn som inte har sjukdomen. Detta talar för att barn med oupptäckt celiaki har nytta av att tidigt bli funna och kunna bli behandlade och därmed att man bör överväga screening av alla svenska barn med genetisk risk.

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