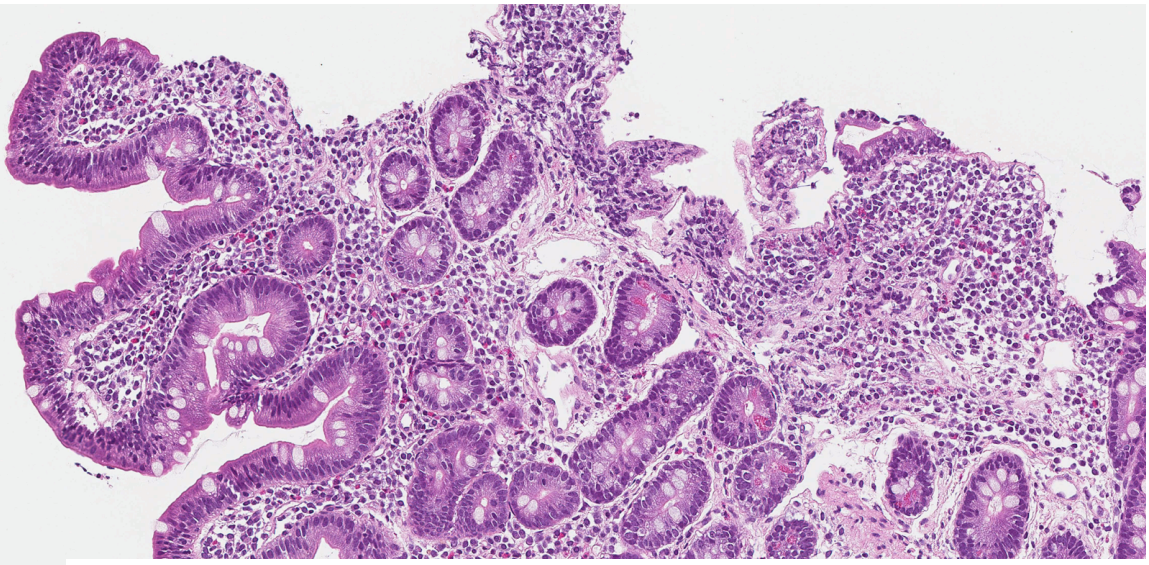
LUND UNIVERSITY

Clinical Aspects of Screening Detected Celiac Disease among 12-year-olds

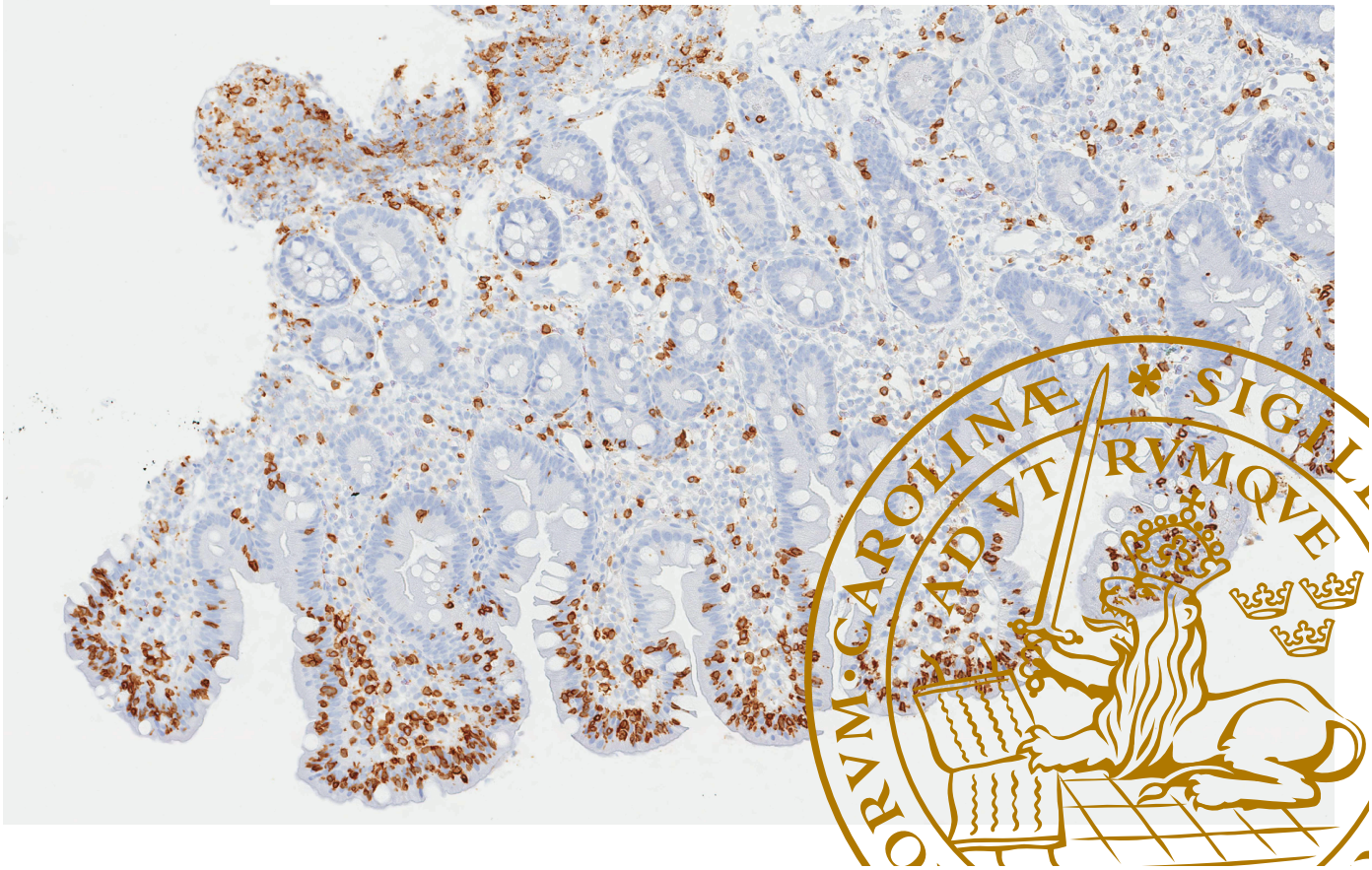
vanderPals, Maria

2015



Clinical Aspects of Screening Detected Celiac Disease among 12-year-olds

MARIA VAN DER PALS
DEPARTMENT OF PEDIATRICS | CLINICAL SCIENCES, LUND | LUND UNIVERSITY



Clinical Aspects of Screening Detected Celiac Disease among 12-year-olds

Maria van der Pals

LUND
UNIVERSITY

Doctoral Dissertation
To be publicly defended, by due permission of the Faculty of Medicine,
Lund University, Sweden, on June 12th, 2015 at 1:00 p.m.
in LUX hörsal del övre, Helgonavägen 3, Lund

Faculty opponent
Professor Jonas Ludvigsson, MD, PhD
Department of Medical Epidemiology and Biostatistics,
Karolinska Institutet, Stockholm, Sweden

Organization LUND UNIVERSITY Department of Pediatrics Clinical Sciences, Lund	*Document name* *Date of issue* May 22, 2015
Author(s) Maria van der Pals	*Sponsoring organization*
Title and subtitle Clinical Aspects of Screening Detected Celiac Disease among 12-year-olds	

Abstract

Sweden experienced an epidemic (1984-96) of celiac disease in children, partly attributed to changes in infant feeding. Our aim was to compare the total prevalence of celiac disease in two birth cohorts of 12-year-olds and relate the findings to each cohort's ascertained infant feeding. Furthermore, we compared the growth parameters in the children with screening-detected celiac disease with their healthy peers. We also investigated the association of thyroid autoimmunity and thyroid function in the celiac disease cases (treated and untreated) compared to the children without celiac disease.

In order to study these questions, a two-phase, epidemic and post-epidemic, cross-sectional screening study was performed. All celiac disease cases, previously diagnosed and screening-detected, were identified. Infant feeding practices were ascertained. Weight, height and BMI were measured and cut-off points recommended by the International Obesity Task Force, used. A case-control study was designed to investigate thyroid autoimmunity (assessed with antibodies against thyroid peroxidase, TPO Abs) and thyroid function (assessed with thyroid stimulating hormone, TSH, and free thyroxine, fT4).

We found a significant difference in prevalence of celiac disease, 2.9% vs. 2.2%, 1993 vs. 1997 cohorts, respectively. The cohorts differed in infant feeding. Screening-detected celiac disease children weighed less and were shorter than their peers. Median BMI was slightly lower. Still, there was no association between being underweight and the risk of having undiagnosed celiac disease and 14% was overweight. Among the previously diagnosed celiac disease cases, 7.5% were TPOAb positive and among screening-detected cases, 7.0%, compared to 2.8% of the controls. Hypothyroidism was more common in children with celiac disease and TPO Abs positivity than in children without celiac disease but with TPO Abs. Positive TPO Abs titer increased the risk of developing hypothyroidism in all groups.
In conclusion, to gradually introduce gluten-containing foods from four months of age, preferably during ongoing breastfeeding, seems favorable. Growth parameters are slightly affected in children with screening-detected celiac disease but not reliable in predicting celiac disease. Even if well treated, celiacs have a higher prevalence of thyroid autoimmunity than their healthy peers. Having TPO Abs in addition to celiac disease, increases the risk of thyroid dysfunction.

Key words Children; Celiac disease; Thyroid autoimmunity; Gluten; Body Mass Index		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		*Language* English
ISSN and key title 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2015:72		*ISBN* 978-91-7619-151-4
Recipient's notes	*Number of pages* 133	*Price*
	Securi	

Signature  *Date* May 15, 2015

Clinical Aspects of Screening Detected Celiac Disease among 12-year-olds

Maria van der Pals

LUND
UNIVERSITY

Department of Pediatrics
Clinical Sciences, Lund
Faculty of Medicine

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2015:72
ISBN 978-91-7619-151-4
ISSN 1652-8220-

Printed in Sweden by Media-Tryck, Lund University, Lund 2015

To my family

Table of contents

List of abbreviations

AGA	Antigliadin antibodies
BMI	Body Mass Index
CI	Confidence Interval
anti-DGP	Anti-deamidated gliadin peptide
EMA	Endomysial antibodies
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology and Nutrition
ETICS	Exploring the Iceberg of Celiacs in Sweden
HLA	Human leukocyte antigen
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IEL	Intraepithelial lymphocytes
OR	Odds Ratio
SD	Standard Deviation
TG2	Tissue transglutaminase 2
tTG	Tissue transglutaminase (refers to TG2 in this thesis)
TPOAb	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
fT4	Free thyroxine

List of publications

I. Ivarsson A*, Myleus A*, Norstrom F, **van der Pals M**, Rosen A, Hogberg L, Danielsson L, Halvarsson B, Hammarroth S, Hernell O, Karlsson E, Stenhammar L, Webb C, Sandstrom O, Carlsson A: Prevalence of childhood celiac disease and changes in infant feeding. *Pediatrics* 2013, 131(3):687-694. *Authors contributed equally

II. **van der Pals M**, Myleus A, Norstrom F, Hammarroth S, Hogberg L, Rosen A, Ivarsson A, Carlsson A: Body mass index is not a reliable tool in predicting celiac disease in children. *BMC pediatrics* 2014, 14:165.

III. **van der Pals M**, Ivarsson A, Norstrom F, Hogberg L, Svensson J, Carlsson A: Prevalence of thyroid autoimmunity in children with celiac disease compared to healthy 12-year olds. *Autoimmune diseases* 2014, 2014:417356.

IV. **van der Pals M**, Norstrom F, Myleus A, Isaksson A, Ivarsson A, Hammarroth S, Hogberg L, Carlsson A: Thyroid function and thyroid autoimmunity in children with celiac disease compared to their healthy peers. *Submitted to Journal of Pediatrics*

All previously published papers were reproduced with permission from the publisher. The papers are referred to by their Roman numerals in the text.

Abstract

Sweden experienced an epidemic (1984-96) of celiac disease in children, partly attributed to changes in infant feeding. Our aim was to compare the total prevalence of celiac disease in two birth cohorts of 12-year-olds and relate the findings to each cohort's ascertained infant feeding. Furthermore, we compared the growth parameters in the children with screening-detected celiac disease with their healthy peers. We also investigated the association of thyroid autoimmunity and thyroid function in the celiac disease cases (treated and untreated) compared to the children without celiac disease.

In order to study these questions, a two-phase, epidemic and post-epidemic, cross-sectional screening study was performed. All celiac disease cases, previously diagnosed and screening-detected, were identified. Infant feeding practices were ascertained. Weight, height and BMI were measured and cut-off points recommended by the International Obesity Task Force, used. A case-control study was designed to investigate thyroid autoimmunity (assessed with antibodies against thyroid peroxidase, TPO Abs) and thyroid function (assessed with thyroid stimulating hormone, TSH, and free thyroxine, fT4).

We found a significant difference in prevalence of celiac disease, 2.9% vs. 2.2%, 1993 vs. 1997 cohorts, respectively. The cohorts differed in infant feeding. Screening-detected celiac disease children weighed less and were shorter than their peers. Median BMI was slightly lower. Still, there was no association between being underweight and the risk of having undiagnosed celiac disease and 14% was overweight. Among the previously diagnosed celiac disease cases, 7.5% were TPOAb positive and among screening-detected cases, 7.0%, compared to 2.8% of the controls. Hypothyroidism was more common in children with celiac disease and TPO Abs positivity than in children without celiac disease but with TPO Abs. Positive TPO Abs titer increased the risk of developing hypothyroidism in all groups.

In conclusion, to gradually introduce gluten-containing foods from four months of age, preferably during ongoing breastfeeding, seems favorable. Growth parameters are slightly affected in children with screening-detected celiac disease but not reliable in predicting celiac disease. Even if well treated, celiacs have a higher prevalence of thyroid autoimmunity than their healthy peers. Having TPO Abs in addition to celiac disease, increases the risk of thyroid dysfunction.

Summary in Swedish

Populärvetenskaplig sammanfattning

Tarmsjukdomen celiaki, glutenintolerans, är en av våra vanligaste kroniska sjukdomar som utvecklas då man inte tål ämnet gluten, ett protein som framför allt finns i vete, råg och korn. För att utveckla celiaki krävs en genetisk sårbarhet, vilket innebär att man i arvsmassan bär på en benägenhet som gör att man kan utveckla sjukdomen vid intag av mat innehållande gluten. Celiaki räknas således som en ärftlig, autoimmun sjukdom. I normalfallet är vårt immunförsvar mycket väl utvecklat. Samtidigt som immuncellerna måste vara redo att snabbt identifiera och neutralisera farliga mikrober som till exempel virus och bakterier, får de inte angripa våra kroppsegna molekyler. För att inte angripa kroppens egna vävnader genomgår immuncellerna en utmognad där de immunceller som reagerar på våra egna molekyler förstörs. Hos vissa individer går denna utbildning fel och immunförsvaret angriper den egna vävnaden och vi utvecklar en så kallad autoimmun sjukdom som till exempel celiaki. Exempel på andra autoimmuna sjukdomar är typ I diabetes, struma (sköldkörtelrubbningar), reumatoid artrit och multipel skleros. Många gånger finns det ett samband mellan olika autoimmuna sjukdomar och detta innebär att celiaki är vanligare i vissa riskgrupper t.ex. hos diabetiker och personer med struma. Dessa sjukdomar har också gemensamt att de orsakas av både genetiska faktorer och vår omgivning och livsstil. Det som dock särskiljer celiaki ifrån andra autoimmuna sjukdomar är att det krävs närvaro av utifrån tillfört gluten för att trigga igång den immunologiska processen som i sin tur leder till en inflammation i tunntarmen. Symptomen på celiaki kan vara mycket varierande. Hos små barn är den s.k. klassiska celiakin mer vanlig med symtom som diarrébesvär, dålig tillväxt och missnöje. Hos äldre barn är diffusa magbesvär, förstoppning och trötthet vanliga och hos vuxna kan lågt blodvärde vara ett tecken. Det är också vanligt att inte ha några symtom alls och då har man till exempel upptäckt sjukdomen vid en screening. Vid misstanke om celiaki kontrolleras glutenantikroppar i blodet och om dessa är förhöjda går man vidare med en gastroskopi där man tar vävnadsprov ifrån övre delen av tunntarmen. Sjukdomen syns i tarmen i form av en slät tarmslemhinna där tarmludden är borta och näringen inte kan tas upp tillräckligt. Om antikropparna i blodprovet är mycket höga, om patienten har symtom och också bär på någon av de speciella riskgenerna är diagnosen celiaki säker och man kan avstå från vävnadsprov. Den enda behandlingen idag är att helt avstå från gluten. Den glutenfria kosten får slemhinnan att läka och bli normal igen.

På befolkningsnivå brukar man tala om ett isberg när det gäller celiaki. Ovanför vattenytan finns de som har fått diagnosen celiaki efter att ha sökt sjukvården med symtom men under vattenytan finns ett stort mörkertal, dvs. de som har celiaki men ännu inte fått diagnos. Denna avhandling består av fyra delarbeten och bakgrunden till arbetena är ETICS studien som betyder ”Exploring the Iceberg of Celiacs in Sweden”. Vi genomförde en screening för celiaki på 12-åringar födda 1993 samt 1997. Bakgrunden till att vi undersökte barn födda dessa årtal är att vi i Sverige har haft en epidemi av celiaki med en ökning under 1980-talet där små barn insjuknade i en klassisk sjukdomsbild. Förekomsten av celiaki var då mycket hög för att sedan minska igen ungefär tio år senare. Man misstänkte att förändringar i spädbarnskosten var en bidragande orsak till epidemin. Skillnaden i spädbarnskosten mellan födelsekohorterna (1993 och 1997) utgjordes huvudsakligen av tre förändringar. Den första var att under epidemin innehöll välling och gröt en betydligt större mängd gluten, den andra var att rekommendationerna angående ålder för introduktion av gluteninnehållande föda senarelades till 6 månader och den tredje var att amningslängden under epidemin var kortare (63 % ammade vid 6 månaders ålder jämfört med 77 % efter epidemin). De två sistnämnda förändringarna innebar att fler barn introducerades till vanlig föda utan pågående amning. Tidigare studier har visat att introduktion av gluteninnehållande föda under pågående amning till viss del kan skydda mot celiaki. De nya rekommendationerna som kom efter epidemin, 1996, blev istället att man kunde introducera gluten gradvis redan från 4 månaders ålder och gärna under skydd av amning vilket sannolikt har bidragit till nedgången av celiaki.

I ETICS-studien ville vi ta reda på mer om vad som kan ha bidragit till denna epidemi och därför var det nödvändigt att jämföra förekomsten av celiaki hos barn födda under respektive efter epidemin. Totalt lämnade 12632 barn blodprov, 7208 (72 % av de inbjudna) i första fasen och 5424 barn i andra fasen (65 %). Vi undersökte antikroppar tydande på celiaki i blodprov och de med tidigare diagnosticerad celiaki rapporterades. Barn med förhöjda antikroppar kallades till barnklinik för vidare undersökning med tunntarmsbiopsi samt genetisk kontroll innan diagnos ställdes. I första delarbetet fann vi genom screeningen att totala celiakiförekomsten bland 12-åringar födda under epidemin (1993) var 2.9%. Detta är den högsta förekomsten som rapporterats i Europa och USA. Vidare fann vi att den totala förekomsten i födelsekohorten efter epidemin (1997) var 2.2% vilket var statistiskt signifikant lägre i jämförelse med under epidemin. I båda födelsekohorterna var celiaki vanligare bland flickor än pojkar och två av tre fall var tidigare odiagnostiserade och hittades genom screeningen. Utöver blodprovstagningen fick föräldrar till de deltagande barnen via enkäter svara på vilken

spädbarnskost de haft. Där kunde vi se att barnen födda under epidemin i större utsträckning introducerats till större mängder gluten efter att amningen avslutats. Våra resultat tyder på att den spädbarnskost som barn födda 1997 fått är mer gynnsam vad gäller risken att utveckla celiaki. Enligt vad vi vet har inga andra stora förändringar vad gäller miljö- och livsstilsfaktorer skett under den här perioden, förutom förändringarna i spädbarnskosten. Generellt sett har autoimmuna sjukdomar och allergier etc. ökat i samhället under de senaste årtiondena av oklar anledning. Detta går i linje med den höga förekomsten av celiaki hos barnen födda 1997 men det förklarar inte varför förekomsten hos barnen födda 1993 var ännu högre.

Utöver att undersöka hur man kan arbeta preventivt för att förhindra celiaki bland befolkningen, var vi också intresserade av att ta reda på om faktorer såsom vikt och längd kan förutsäga celiaki hos barn. Som tidigare beskrivits finns ett stort mörkertal vad gäller förekomsten av celiaki och många barn har sjukdomen utan att veta om det. I andra delarbetet undersökte vi skillnaderna i vikt, längd och BMI (body mass index som visar relationen mellan vikt och längd) hos 12-åringar med screening-upptäckt, obehandlad celiaki och jämförde dessa med deras friska kamrater. Vi upptäckte att 4.2% av barnen med celiaki var underviktiga jämfört med 5.2 % av de friska barnen, 82 % var normalviktiga jämfört med 72.8 % av de friska barnen och endast 13.8 % av barnen med celiaki var överviktiga jämfört med 21.9 % av friska 12-åringar. Sammantaget var majoriteten av barnen med screening-upptäckt celiaki normalviktiga men som grupp betraktat vägde 12-åringarna med celiaki generellt sett mindre och var något kortare jämfört med sina kamrater. Det är således viktigt att inte gå med oupptäckt, obehandlad celiaki för länge. Dock kan man inte använda sig av vikt och längd hos den enskilde för att säga vem som har celiaki eller inte. Även om celiaki är mindre vanligt bland överviktiga barn så kan man hitta sjukdomen även bland dessa barn.

Vid förekomst av celiaki är det känt att risken är större att utveckla andra autoimmuna sjukdomar, bland annat sköldkörtelsjukdomar som struma. Man vet dock inte om man kan förhindra detta genom att sköta den glutenfria kosten väl och sålunda ha en välbehandlad sjukdom. I de tredje och fjärde delarbetena ville vi bland de deltagande 12-åringarna undersöka associationen mellan kliniskt upptäckt, behandlad, celiaki och kopplingen till sköldkörtelpåverkan jämfört med screening-upptäckt, obehandlad, celiaki och sköldkörtelpåverkan. Vi ville vidare undersöka hur många av barnen med celiaki som hade påverkan på sköldkörteln jämfört med de friska 12-åringarna. Ett tecken på påverkan i sköldkörteln kan vara förekomsten av så kallade autoantikroppar,

dvs. antikroppar riktade mot kroppens egna vävnader. Vi undersökte en av dessa antikroppar som kallas TPO (thyroidea peroxidas) där förhöjda värden kan ses vid kronisk inflammation i sköldkörteln. Vi fann i studien att förekomsten av sköldkörtelpåverkan mätt i form av antikroppar mot TPO var nästan tre gånger högre hos barnen med celiaki jämfört med de friska 12-åringarna. Det var ingen skillnad om man hade en välbehandlad celiaki sedan tidigare jämfört med en obehandlad celiaki som upptäckts genom screeningen. Att ha TPO antikroppar utöver celiaki ökade också risken att ha en rubbning i själva sköldkörtelfunktionen där den vanligaste förekommande rubbningen var en underfunktion i sköldkörteln. De flesta hade dock en mild form av underfunktion och det verkar idag som att detta tillstånd är relativt beskedligt. Det är inte säkert att det fortskrider till en behandlingskrävande sköldkörtelsjukdom. Dock behöver man följa barnen under en längre tid även upp i vuxen ålder för att ta reda på detta, eftersom rubbningar i sköldkörtelfunktionen är betydligt vanligare bland vuxna än bland barn.

Sammanfattningsvis har vi visat att celiaki är betydligt vanligare bland barn i Sverige än man tidigare trott och majoriteten av dessa fall är odiagnostiserade. Vi fann även en signifikant skillnad i den totala förekomsten av celiaki (hela isberget) mellan barnen födda under jämfört med efter epidemin. Sålunda har vi med denna avhandling visat att celiaki kan förhindras hos vissa individer, åtminstone upp till 12-års ålder. Vi har vidare visat att det inte går att förutsäga vem som har celiaki utifrån att titta på barnets längd och vikt eftersom celiaki förekommer hos alla barn oavsett kroppskonstitution. Det är dock viktigt att hitta obehandlad celiaki eftersom den gruppen som helhet är något mindre än sina jämnåriga kamrater. Likaså är det viktigt att ta reda på om barnet har celiaki eftersom förekomsten av andra autoimmuna sjukdomar är högre hos dessa barn. I den här avhandlingen såg vi att sköldkörtelpåverkan var tre gånger vanligare. Det verkade dock inte spela någon roll om barnet hade behandlad eller obehandlad celiaki när det gällde att utveckla sköldkörtelpåverkan. Våra resultat styrker snarare att det är gemensamma gener som står för samsjukligheten. Även om det inte är säkert att det går att förhindra utvecklingen av andra autoimmuna sjukdomar genom att äta glutenfri kost är det viktigt att trots det veta om att barnet har celiaki. Detta då det gör att man kan vara mer uppmärksam på andra tillstånd som sköldkörtelrubbning som likt celiaki kan yttra sig som enbart diffusa symtom som till exempel trötthet.

Thank you/Tack

Många människor har varit inblandade som jag vill tacka för att den här avhandlingen var möjlig att genomföra. Först och främst vill jag rikta ett stort tack till alla deltagande barn och familjer i ETICS studien. Tack för att ni ställer upp och hjälper oss att komma lite närmare celiakins gåta! Tack till alla medarbetare, sjuksköterskor, skolpersonal och laboratoriepersonal och alla andra som gjort screening-studierna genomförbara.

Jag vill tacka min huvudhandledare, **Annelie Carlsson**, för din hjälpsamhet, din uppmuntran och ständiga tro på mig. Du är så bra för självförtroendet!

Tack också till mina bihandledare, **Anneli Ivarsson** och **Anna Myleus**, för stöd och vetenskapliga råd. Ett särskilt tack till dig, **Anna**, för att du alltid är så hjälpsam och stöttande och att du alltid tar dig tid att reda ut saker som jag hängt upp mig på – du är så bra på att förklara och se samband!

Tack också till alla övriga forskarkollegor i **ETICS** gruppen, både uppe i Umeå och här nere i Skåne. Tack, **Fredrik Norström**, för att du är ett bra bollplank när det gäller statistiken. Särskilt tack också till **Lars Stenhammar** för värdefulla synpunkter på min text!

Tack till **Bengt Lindberg** och **Katarina Johnsson**, för att jag har fått möjlighet till forskningstid under detta år. Tack till alla mina trevliga kollegor på barnkliniken, SUS, Malmö och Lund, särskilt inom gastrosektionen, **Irene Axelsson**, **Pia Karlsland-Åkeson** och **Sara Björck**, för att ni ställt upp under våren och dragit ett tungt lass när jag haft forskningstid. Kul att kunna snacka av sig med varandra, **Sara**, och nu är det snart din tur!! Tack till all personal på **barnmottagningen** och **dagvården** och särskilt tack till **Anna Andersson** och **Lotta Söderberg** för go vänskap och för att ni varit så bra ”stand in-doktorer”, det går ju lika bra!

Tack, **Daniel Agardh**, för din energi och framåtanda och ditt eviga ’kämpande’ med mig inom skopin och bedömning av tunntarmsslemhinnan. Din inställning att ”ingenting är omöjligt” smittar av sig!

Tack, **Gabriele Wurm-Johansson**, för att det är så roligt att skopera med dig och för din hjälpsamhet och tack till all övrig **personal på endoskopi och ORK**. Tack för gott samarbete!

Jag vill också nämna alla goa vänner utanför (och även inom) jobbet som sett till att jag fått mysigt och trevligt umgänge utanför datorn även om jag varit ganska asocial just den här våren! Och till alla mina kitevänner, längtar ut på riktiga havet så fort detta har rotts i land!! And not the least, our American friends, we miss you! Looking forward to seeing you soon!!

Tack till mina svärföräldrar, **Monica** och **Stefan van der Pals**, som sett till att barnen fått det trevligt under helger som vi har jobbat och ett särskilt tack till farfar Stefan för hjälp med körningar till barnens träning! Och till hela släkten van der Pals - sommarens resa kommer att bli så välbehövlig!

Tack till min käre bror och svägerska och våra goa kusiner, **Andreas**, **Katarina**, **Arvid** och **Harald Fagher**! För att vi alltid har så himla roligt ihop!

Mitt varmaste och innerligaste tack till mina fantastiska föräldrar, **Klas** och **Margaretha Olsson**, som alltid ställer upp i vått och torrt! Jag vet inte hur jag ska kunna tacka er för allt ni gör för oss så jag skriver som barnen brukar säga ”Tack för allt!”.

Till sist vill jag tacka min familj, **Jesper**, **Isak** och **Jakob**, för att det är ni som ger livet mening och glädje! Utan er hade inget varit meningsfullt!

Background

Celiac disease is one of the most common chronic diseases in childhood and affects approximately 0.5%–3% of the population in the Western world [1-3]. It is characterized by an autoimmune response triggered by gluten and other environmental cofactors that leads to small-intestinal mucosal injury [4]. The disease can have its onset at any age throughout life, and its clinical expression is heterogeneous. The classic presentation of celiac disease is commonly described as diarrhea, abdominal distention, malnutrition, and failure to thrive [5-7].

History

Celiac disease was first described by Aretaeus of Cappadocia who is considered to have been one of the best clinical physicians of the Ancient World. Aretaeus of Cappadocia was active in Anatolia in the 1st century CE, during a period of rapid development, when agriculture had spread to the so-called region of the Fertile Crescent in the Middle-East [8]. His "Cœliac Affection" (*coeliac* from Greek κοιλιακός *koiliakos*, "abdominal") gained the attention of Western medicine when Francis Adams presented a translation of Aretaeus's work (Adams, F, The Extant Works of Aretaeus the Cappadocian) in London, The Sydenham Society, 1856. In modern time, the pediatrician Samuel Gee first described the condition in children in a lecture at the Hospital for Sick Children, Great Ormond Street, London, in 1887. Gee adopted the same term as Aretaeus (celiac disease). He insightfully stated: "If the patient can be cured at all, it must be by means of diet" even if he failed to identify the food causing the disorder. However, he recognized that milk intolerance is a problem and he highlighted particular success with a child "who was fed upon a quart of the best Dutch mussels daily" [7-10]. In the beginning of the 20th Century, a few other researchers tried to find food items causing the disease but the breakthrough did not come until the Dutch researcher Willem Dicke made a discovery a few years later. Dicke believed that wheat was the cause already in the 1930s but it was not until the Dutch famine 1944 when the mortality dropped substantially due to the shortage of bread. These findings led to his doctoral thesis a few years later at the University of Utrecht where he showed that exclusion of wheat, rye and oats from the diet led to dramatic improvement in the general condition of the child and marked reduction in the fatty diarrhea [11]. A couple of years later, researchers found that gluten was the main substance causing the disease [12, 13]. Diagnosis was made by intestinal biopsy with the Crosby-Kugler capsule and the typical histological

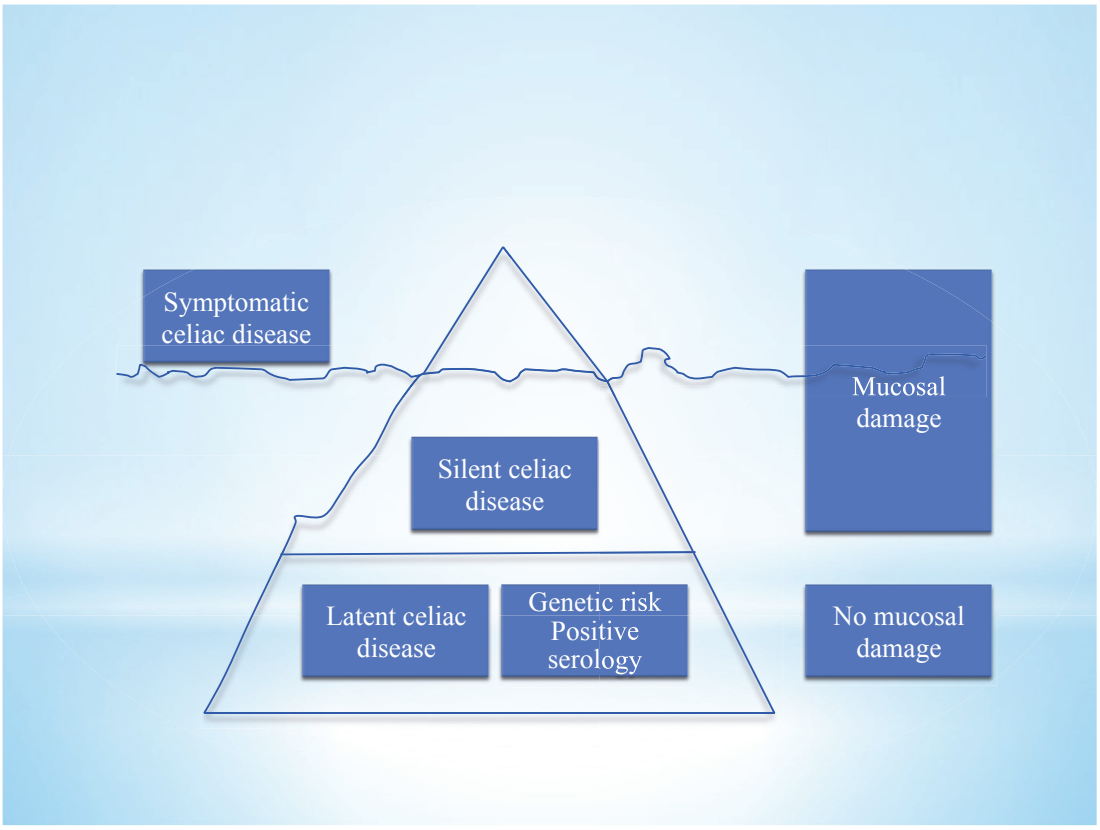


Figure 1. The Celiac Iceberg with the tip of the iceberg showing the symptomatic celiac disease cases.

mucosal damage with crypt hypertrophy and flat mucosa was found to constitute the pathogenesis of the intestinal damage in celiac disease. In 1954, Paulley reported that the clinical manifestations of celiac disease are linked to destruction of the lining of the small intestine which many years later was classified histologically according to Marsh [14-16]. In the 60s, celiac disease was thought of as an autoimmune disease, associated conditions such as dermatitis herpetiformis was found and the connection with short bowel malignancy and the identification of antigliadin antibodies (AGA) fundamentally changed the perspective of celiac disease. The immune response rather than the protein itself was found to be the cause of the mucosal damage [17]. As a result of the discovery of AGA, the connection between celiac disease and other autoimmune diseases such as type 1 diabetes was established, linked to sharing of HLA genes (please see *Genetics* section below for further information). Increasingly more autoantibodies were found; reticulin 1971, and later endomysium antibodies in monkey esophagus and then human umbilical cord section [18-20]. The endomysium is a structure of the smooth muscle connective tissue. It was later discovered that the target antigen

in endomysium is tissue transglutaminase (tTG) and gliadin, a component of wheat gluten is a preferred substrate [21]. Ludwig Sollid hypothesized a model linking gluten to tTG and to anti-tTG autoantibodies. The same research group showed that tTG deaminates gluten-derived peptides and increases their affinity to the DQ2 and DQ8 HLA, thus worsening the consequences of anti-gluten immunity [22].

During the 1990s, the idea of the "Celiac Iceberg" was presented and celiac disease developed from being seen as an unusual bowel disease due to gluten, expressed with gastrointestinal symptoms (the tip of the iceberg), to a common autoimmune disorder triggered by gluten in the gut but with a clinical expression involving a variety of symptoms from different organs [23], **Figure 1**.

Pathogenesis

Genetics

Celiac disease has a strong hereditary component and genetic susceptibility is a prerequisite to develop celiac disease. There is a strong association with human leukocyte antigen (HLA) [24]. The genes encoding for HLA molecules are found in the major histocompatibility (MHC) complex on chromosome 6. The primary function of class II molecules is to bind peptide antigens and present them to a T cell receptor on CD4 (helper) T cells. B and T cells normally recognize self-antigens as 'safe' and it does not lead to an immune response. Similarly, oral administration of antigen leads to immune tolerance by the means of anergy, immunologic tolerance, which refers to the failure to mount a full immune response against a target. Such oral tolerance fails for gluten (and certain other prolamin) peptides in celiac disease at which point a T cell response is engaged [25]. MHC class II molecules consist of an α and β chain and they are encoded by three different loci, HLA-DR, -DQ, and -DP, which display ~70% similarity to each other. DQ2 and DQ8 are linked to celiac disease [26, 27]. HLA-DQA1 genes code for two α-chains (α1 and α2) and HLA-DQB1 genes code for two β-chains (β1 and β2). These are associated as heterodimers on the surface of antigen presenting cells and form a cleft that binds antigens [28]. HLA-DQ2 is encoded by the HLA-DQA1*05 allele (α-chain) and HLA-DQB1*02 allele (β-chain). The two alleles are often present in the cis conformation on the DR3 HLA-DQA1*0501-DQB1*0201 haplotype (HLA-DQ2.5cis), which is also common to many other autoimmune disorders [29]. They can also be encoded in trans on the HLA-DQA1*0505-DQB1*0301/ DQA1*0201-DQB1*0202 haplotypes (HLA-DQ2.5trans), **Figure 2**.

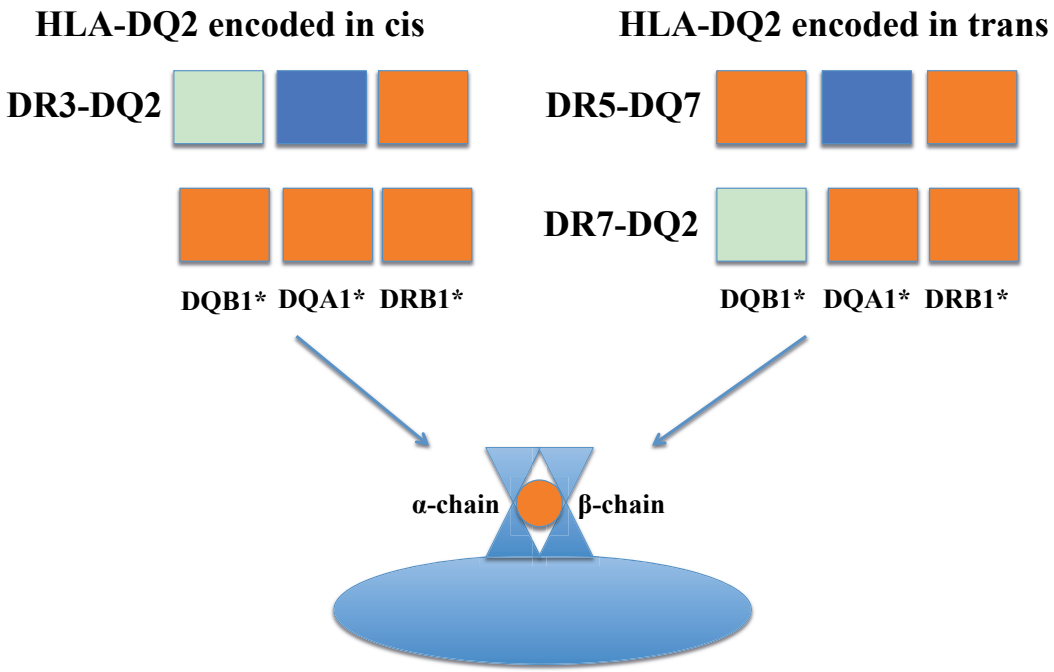


Figure 2. HLA-DQ2 is encoded by the HLA-DQA1*05 allele (α-chain) and HLA-DQB1*02 allele (β-chain). The two alleles are often present in the *cis* conformation on the DR3 HLA-DQA1*0501-DQB1*0201 haplotype (HLA-DQ2.5cis). They can also be encoded in *trans* on the HLA-DQA1*0505-DQB1*0301/ DQA1*0201-DQB1*0202 haplotypes (HLA-DQ2.5trans).

DQ2.5 heterozygotes are the most common HLA configurations and represent up to 50% of the HLA types found in celiac disease patients [30]. Disease susceptibility depends on the dosage effect of the DQ2.5 heterodimer. One of the DQA1*0501 and DQB1*0201 alleles appears to be sufficient for conferring susceptibility to celiac disease [25, 26]. However, the highest risk for developing celiac disease are those that display DQB1*02 homozygosity (carrying DQB1*02 on both chromosomes) [31-33]. In a recently performed large international study, homozygosity for HLA haplotype DR3–DQ2, conferred the highest risk of celiac disease autoimmunity and celiac disease and was associated with the earliest onset [34].

DQ8 is a heterodimer composed of α-chains encoded by DQA1*0301 and β-chains encoded by DQB1*0302. In celiac disease overall, HLA-DQ8 is found in 5–10% of patients [27, 30]. A dosage effect for DQ8 molecules has also been suggested [27].

Celiac disease patients not having any of these above mentioned HLA genes are a rarity, only approximately 0.4% [27]. The frequency of celiac risk HLA genotypes in the

general population is approximately 30-40% but higher frequencies have recently been reported [34, 35]. However, only 1–3% actually develop the disease. As the heritability of celiac disease is estimated to be 80% and HLA is estimated to contribute 35-40% of the genetic risk there must be more genetic risk factors involved in celiac disease susceptibility [36, 37]. Twin studies show a large discrepancy in the concordance rate of celiac disease in monozygotic twins (75%) compared to HLA-identical dizygotic twins (11%) [38, 39]. Thus, HLA genes are important, but not sufficient to predispose to celiac disease. Genome-wide association studies (GWAS) so far allowed the identification of 39 non-HLA CD-associated loci that together contain 115 different genes. Most of them control T-cell activation and recruitment, pointing to an altered immune system response underlying celiac disease. However, individually each of these non-HLA genes is believed to play a relatively small role in celiac disease [36]. The 39 non-HLA loci could explain nearly 14% of the genetic variance of celiac disease, with the HLA locus accounting for another 40% as mentioned earlier. Thus, much heritability in celiac disease still remains unknown.

Gluten as the trigger and the subsequent immunologic response

Autoimmune disease affects as many as 5% of the people in the Western world [40]. *Autoimmunity* can lead to autoantibodies, antibodies against host antigens, or to autoreactive T cells, lymphocytes. *Autoimmune disease* is described as an abnormal immune response, directed against antigenic components of the host mediated by the pathogenic autoantibodies or the autoreactive T cells (cell-mediated autoimmune disease). Despite the fact that gluten, and not a self-antigen, is the active cause, celiac disease can be viewed as an organ-specific autoimmune disease.

The normal immune response: The immune responses usually result from the combined effects of antigen-specific stimuli on the immune system and of antigen-non-specific activation of antigen-presenting cells processes [41]. Regulatory mechanisms usually limit the development of autoimmune responses. There are numerous mechanisms involved in maintaining the homeostasis and balancing immune responses directed to eliminate invading pathogens with the need to minimize collateral damage to the tissue as a result of a defect immune response [42]. Dendritic cells (DCs) are central to the process in deciding whether a T cell becomes activated or deleted and this control takes place during T cell priming in lymphoid organs. If the T cell becomes activated, DCs influence what type of effector or regulatory functions it will gain [43, 44]. DCs regulatory properties are influenced by the signals they receive from the tissue

environment and the intestinal dendritic cells gain immune regulatory properties from the gut environment and imprint gut-homing specificity on T cells. Another control occurs in the tissue, called 'licensing', where cytotoxic T lymphocytes (CTLs) receive necessary signals to become functional effector and memory cells or become resistant to the inhibitory effects of regulatory T cells [45, 46].

As mentioned before, one of the specific characteristics for celiac disease as an autoimmune disease is that the antigen that triggers the abnormal immune response is a non-self antigen. Gluten ingestion is a prerequisite to develop celiac disease and as described above, genetic susceptibility is another prerequisite for celiac disease as for many other autoimmune disorders.

Gluten

Wheat is widely consumed by humans and is one of the three most important crops in the world, together with maize and rice [47]. Wheat contributes essential amino acids, minerals, and vitamins, and beneficial phytochemicals and dietary fiber components to the human diet, and these are especially enriched in whole-grain products.

The main route of wheat being introduced into Europe was via Anatolia to Greece (8000 BP) and then through the Balkans to the Danube (7000 BP) and across to Italy, France and Spain (7000 BP), reaching the UK and Scandinavia by about 5000 BP. Wheat spread via Iran into central Asia reaching China by about 3000 BP and to Africa, initially via Egypt. It was introduced to Mexico in 1529 and to Australia in 1788. Despite wheat being a relatively 'new' food source, bread wheat shows sufficient genetic diversity to allow the development of over 25 000 types which are adapted to different environments [48].

The protein content of wheat varies from about 10–15% of the grain dry weight, with about half of the total being storage proteins [49]. The gluten protein fraction brings the viscoelastic properties essential for dough formation of wheat flour and makes it the base for bread, noodles, pasta, and other food products, widely used in the food industry. The large majority of the proteins are of a single type called prolamins. If dough is washed, a proteinaceous mass is left that is highly elastic. Gluten comprises approximately 75% protein on a dry weight basis, with most of the remainder being starch and lipids [47]. As a consequence of its widespread usage in the food industry, the daily gluten intake on a gluten-containing diet in the United States and Western Europe is high, between 15 and 20 g per day [50].

Gluten is a heterogeneous mixture of gliadins and glutenins in wheat or similar proteins in barley and rye, called hordeins and secalins, respectively [51]. The avenins in oats are homologous to gluten but much less so than the hordeins and secalins [52]. Gluten has a very high content of the amino acids glutamine (30%) and proline (15%) and because of its high proline content, the 33-mer gliadin peptide is highly resistant to degradation by gastrointestinal enzymes. Thus, large immunogenic gluten peptides will reach the gut mucosa. Long, proteolytically resistant fragments were widespread within the α-gliadin, γ-gliadin, glutenin, hordein and secalin protein families of wheat, rye and barley. In contrast, investigations on sequences of avenins from oats showed no peptide products longer than 10 amino acids and their susceptibility to proteolysis before reaching the gut mucosa, corresponds to their low proline content, an average of 6% in avenins. Furthermore, these avenin fragments contain T cell epitopes recognized by intestinal T cells of oats-intolerant patients with celiac disease [53, 54]. However, consumption of oat thus results in a much lower exposure to antigenic peptides, in comparison with the other cereals, and this is apparently tolerated, as it does not lead to disease in the majority of patients [50].

The immunologic response

Gluten seems to have a dual effect upon the small bowel mucosa. Under normal, intestinal homeostasis conditions, enterocytes secrete signals and the dendritic cells help to maintain the homeostasis. However, in celiac disease, the intestinal homeostasis system is disrupted. There is a combination of activity by the innate and adaptive immune system in the generation of gliadin-reactive T cells, a cytotoxic response, and autoantibody formation. When gluten is ingested, small toxic peptides, induce an *innate unspecific immunologic response*, characterized by the activation of intraepithelial lymphocytes (IELs) expressing the activating receptor NK-G2D, a natural-killer-cell marker. The majority IELs are CD8+ T cells and in active celiac disease, the number of these cells is markedly increased [50, 55]. The activated lymphocytes become cytotoxic and kill enterocytes and interleukin 15 (IL-15) is involved in this process. The ability of the intestinal epithelial cells to lyse enterocytes contributes to the typical tissue damage in celiac disease [56]. IL-15-dependent NK cell-like transformation of IELs may be an essential step in the immunopathology of refractory celiac disease [50, 51]. This innate immune response will favor enterocyte apoptosis mechanisms and tight junction weakening, together with oxidative and immune stress, and will also inhibit the secretion of homeostatic signals by enterocytes. Within this tissue microenvironment, newly recruited dendritic cells at the lamina propria are not educated into a homeostatic profile.

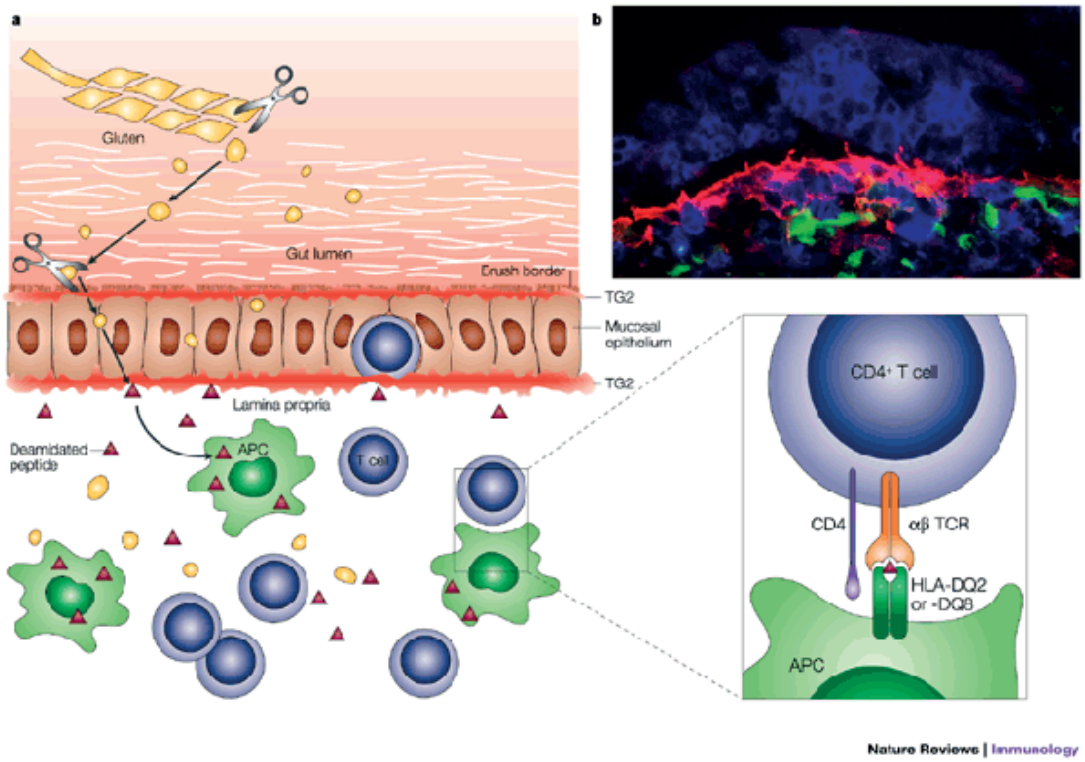


Figure 3 a) The parts of gluten that are resistant to processing by luminal and brush-border enzymes will survive digestion, and can be transported across the mucosal epithelium as polypeptides. Gluten peptides are deamidated by tissue transglutaminase (TG2), which, in the intestinal mucosa, is located mainly extracellularly in the subepithelial region, but is also found in the brush border. $CD4^+$ T cells in the lamina propria recognize deamidated gluten peptides predominantly, presented by HLA-DQ2 or -DQ8 molecules on the cell surface of antigen-presenting cells (APCs). **b)** Immunofluorescence staining of TG2 (pink), HLA-DQ (green) and T cells (CD3; purple) in the small-intestine mucosa of an untreated celiac-disease patient. Note that there is a close spatial relationship between TG2, APCs that express HLA-DQ and T cells just beneath the epithelium. Immunofluorescent image courtesy of H. Scott, Rikshospitalet. Reproduced with permission from Nature Reviews Immunology, copyright © Nature Publishing Group [61].

On the contrary, proinflammatory dendritic cells are generated and capture antigens arriving in the lamina propria for antigen presentation, thus favoring the development of proinflammatory cytokine-secreting lymphocytes that are ultimately involved in tissue injury and the loss of immune tolerance [45]. Tissue injury is also mediated by metalloproteases, produced by stimulated residential stromal and immune cells, and their expression may be activated by Th-1 type cytokines [57]. Interferon (IFN)-α, which is highly expressed in celiac disease mucosa may play a critical role in promoting the differentiation of proinflammatory dendritic cells [58]. The production of IFN-γ is a signature of "gluten" peptide–specific HLA-DQ2– and HLA-DQ8–restricted T cells that are isolated from the mucosa of the small intestine of celiac disease patients,

and it is considered to have a key role in the downstream initiation of mucosal damage [59]. This profile of cytokines is responsible of the epithelial lesion, characterized by the presence of IELs, crypts hyperplasia, villous atrophy, and chronic inflammatory infiltrate at the lamina propria.

The *adaptive immunologic response* is performed by the increased permeability that permits immunologic peptides such as 33-mer to enter the lamina propria. Both HLA-DQ2.5 and HLA-DQ8 favor the binding of peptides with negatively charged amino acids at anchor residues. Gluten peptides, however, are largely devoid of negative charges, and native gluten peptides thus bind poorly to HLA-DQ2.5 or HLA-DQ8. The calcium-dependent enzyme tissue transglutaminase (TG2) can modify gluten peptides to fit the requirements for high affinity binding to HLA-DQ2 and HLA-DQ8 and convert non-charged glutamine into negatively charged glutamic acid, a process called deamidation [21, 22] **Figure 3.** TG2 is mostly retained intracellularly in an inactive form and is activated upon its release during tissue damage [60]. Thus, something must trigger tissue damage which initiates TG2 release, allowing the modification of gluten peptides. The initial CD4+ T cell response may be directed against native gluten peptides and this may be sufficient to induce tissue inflammation and consequently TG2 activation [42]. This could represent the first breach in oral tolerance to gluten and trigger a self-amplifying loop: CD4+ T cell responses against native gluten peptides lead to IFN- production, to upregulation of HLA, and further amplification of the gluten-specific T cell response. TG2 released upon tissue damage increases the gluten peptide load, which will ultimately lead to more tissue damage [50].

Environmental factors

As described earlier, HLA and the 39 non-HLA loci in celiac disease can explain approximately 50% of the variation in celiac disease risk. Among monozygotic twins with celiac disease, 25% never contract the disease. It should be considered that these twins have the same genetics and in addition often have partly similar environmental exposures by being brought up in the same household during the same time period. Thus, it is evident that environmental factors have an important role in the development of celiac disease although many issues are still unsolved. Among those discussed here are *infections, microbiota, breastfeeding* and *gluten introduction* with respect to age and amount and overlap with breastfeeding.

Enteric viruses may affect the permeability of the mucosa in the small intestine and may contribute to disease development in a genetically predisposed individual [51]. High

frequency of rotavirus *infections* may increase the risk of celiac disease autoimmunity in childhood in genetically predisposed individuals [62]. A population-based incident case-referent study showed that repeated infectious episodes, regardless of type of infection, during the first six months of life was associated with a significantly increased risk for later celiac disease. Moreover, a synergistic effect between early infections and daily amount of gluten intake was found, more pronounced among infants for whom breastfeeding had been discontinued prior to gluten introduction [63]. Others have found contradictory results; infections during the time of gluten introduction was not a risk factor for future celiac disease although the authors could not exclude the possibility that specific pathogens constitute risk factors for celiac disease, since the risk estimates for infection at the time of gluten introduction were of borderline statistical significance. No association was found between age at gluten introduction or breastfeeding duration and future celiac disease [64].

Many recently performed studies have focused on trying to understand the relationship between the human gut *microbiota* and celiac disease pathogenesis. Even though some differences in the gut microbiota between the children with celiac disease and healthy have been found, the findings have so far been inconclusive [65-67]. However, it seems as if conditions leading to disturbances in the composition of the gut microbiota early in life can lead to long-term dysbiosis, with an excess of celiac disease-associated bacteria. These abnormal microbiota compositions may influence the amount of proinflammatory cytokines and the responses to gluten and constitute a major risk factor for contraction of celiac disease in genetically at risk children [66].

Breastfeeding influences the composition of the intestinal microbiota and protects against gastrointestinal infections [68]. Breastfeeding during the *introduction of dietary gluten*, and increasing duration of breastfeeding were associated with reduced risk of developing celiac disease [69]. However, introducing foods containing gluten in the first three months of life increases a child's risk of celiac disease autoimmunity. Interestingly, waiting until the seventh month or later to first introduce food containing gluten marginally increases the risk for celiac disease autoimmunity compared with introducing gluten between the age of four and six months [70]. Yet, it was not clear from the primary studies whether breastfeeding delays the onset of symptoms or provides a permanent protection against the disease. A foreign antigen such as a food protein at a specific time interval in infancy could promote immune tolerance. It has been shown that to maintain tolerance to a food that was once responsible for allergy, continual exposure of the immune system to the allergen may be necessary [71]. The introduction of gluten at six months of age is a long-standing practice but in 2001, the World Health

Organisation (WHO) revised its recommendation for exclusive breastfeeding from four to six months [72]. These recommendations were aimed at reducing morbidity in developing countries, but may not be appropriate in the growing world population experiencing progressive industrialization and escalating risk of immune dysregulation. The rising rates of allergic and autoimmune diseases highlight the susceptibility of these tolerance pathways to environmental changes. Although the mechanisms are not clear, many of these conditions (including food allergies, celiac disease and type 1 diabetes) manifest early in life, indicating that immune dysregulation is a very early event [73]. Animal models suggest that tolerance is an antigen (allergen)-driven process and that exposure to these proteins during a critical early window of development may be essential to this process. This also appears to coincide with the establishment of healthy gut colonization, which has been shown to be essential in promoting tolerance to both allergens and self antigens [74]. Delays in either colonization or antigen/ allergen exposure can lead to failure of oral tolerance [70, 71]. Thus, the optimal timing of the introduction of gluten in the infant's diet has still to be investigated.

In this thesis, paper I evaluates the possible relationship between infant feeding and later risk for celiac disease. We investigated and compared the total prevalence of celiac disease, including both previously diagnosed (clinically detected) and screening-detected cases, in two birth cohorts of 12-year-olds that differ regarding infant feeding.

Clinical presentation

Gastro-intestinal and extra-intestinal symptoms

The signs and symptoms of celiac disease can vary greatly and are influenced by age. As mild symptoms are common, the majority of individuals with celiac disease remain undiagnosed [2, 5]. Younger children often present with a classical disease with gastrointestinal symptoms, weight loss, malabsorption and growth retardation [75, 76]. Abdominal pain, vomiting, and constipation are atypical gastrointestinal symptoms more common in older children and teenagers but the clinical presentation seems to have changed in recent decades and the proportions of patients suffering from classical gastro-intestinal symptoms, including weight loss, are decreasing. Many reports indicate that patients with celiac disease instead have a high or normal body mass index (BMI) at diagnosis [77-80]. More patients get celiac disease diagnosed due to extra-intestinal conditions and symptoms such as arthritis, neurological diseases, iron-deficiency, anemia, short stature and delayed puberty [81-83]. The neurological symptoms associated with celiac disease include cerebellar ataxia, idiopathic epilepsy,

peripheral neuropathy, and recurrent headaches [84]. Psychiatric problems including depression and anxiety are especially common in adolescents with celiac disease and evidence suggests that the gluten-free diet may help in alleviating depression in adolescents with celiac disease [85]. Frequent oral aphthous ulcers and dental enamel defects can occur [86], as well as low bone mineral density and osteoporosis [81]. In adults, approximately one-third of patients at their diagnosis of celiac disease have signs of osteoporosis, one-third have osteopenia, and one-third have normal bone mineral density [87]. Many patients have no symptoms and are found in screening studies for example in a child or adolescent with celiac disease-associated conditions [75, 88, 89].

Body Mass Index (BMI) As described above, in contrast to previous beliefs, it is now well known among adult patients that they have normal weight or even overweight. Some studies show that BMI increases on a gluten-free diet, especially in those who adhere closely to the diet [90]. However, other studies describing BMI in individuals at diagnosis of celiac disease and/or after introduction of a gluten-free diet have shown contradictory results. Few studies examining BMI and other growth parameters have been performed in children, and the findings of those studies have been inconclusive [91-93].

The main objective of paper II in this thesis was to examine weight, height, and BMI in 12-year-old children with untreated screening-detected celiac disease and to compare these parameters with their healthy peers.

Associated disorders of celiac disease including thyroid autoimmunity

Celiac patients have an increased rate of autoimmune diseases. A significantly increased prevalence of other autoimmune diseases has been reported in individuals with celiac disease and their first-degree relatives as compared to controls [94-98], with an estimated burden of autoimmune disease in celiac disease cases up to 15% and this compares with 3% in the general population [40]. The diseases include most commonly type 1 diabetes, thyroid diseases, psoriasis, neurologic problems, and autoimmune liver diseases [99]. Celiac hepatitis usually follows a benign course with slightly elevated transaminases but there have been reports that liver failure was reversed with a gluten free diet [81]. In addition, celiac disease has an association with selective IgA deficiency and with chromosomal disorders such as Down syndrome and Turner syndrome [100]. Dermatitis herpetiformis is characterized by an often symmetrical, vesicular appearance

and pruritus. Treatment is with a gluten-free diet and Dapsone, an antibacterial agent, can be used for symptomatic relief of the rash. The diagnosis is likely under-recognized since it is easily mistaken for other more common dermatological disorders [101].

Paper III and paper IV focus on thyroid disorders, as described below.

Thyroid disorders

The association between celiac disease and thyroid diseases such as Hashimoto thyroiditis, has been well established [102-104]. Hypothyroidism occurs in 5%-15% of adult patients with celiac disease [102, 105]. This is about four times greater than the risk of hypothyroidism in controls [97]. Celiac disease occurs in 2%-5% of people with autoimmune thyroid disease, which is significantly more prevalent than controls [106, 107]. The relation between celiac disease and autoimmune thyroid disease has been attributed to a common genetic susceptibility and the association with the gene encoding cytotoxic T-lymphocyte-associated antigen-4, in both diseases. This is a gene known to confer predisposition to thyroid autoimmunity. Furthermore, it has also been shown that tTG-2 IgA antibodies react with thyroid tissue and this could contribute to the coexistence between celiac disease and thyroid disease [106, 108]. In general, autoantibodies are markers of future disease in presently healthy individuals [109]. In particular, antibodies against thyroid peroxidase (TPO Abs) are an early sign of lymphocytic infiltration in the thyroid gland. These antibodies are known to be a hallmark of autoimmune thyroid disease and present in almost all adult patients with Hashimoto's thyroiditis [109-112]. TPO antibodies seem to directly inhibit the enzymatic activity of TPO and cause thyrocyte destruction by complement-mediated and/or antibody-dependent cell-mediated cytotoxicity [111]. In most cases, the immune response to the target cells progressively destroys the endocrine gland, and hypofunction is the main clinical manifestation [111, 113]. Gluten exposure, as in untreated celiac disease, has been suggested to increase the risk of developing these related autoimmune diseases compared to individuals with celiac disease on a gluten free diet [114-116]. However, the role of gluten in associated autoimmune disorders is still unclear and other studies have shown contradictory results [117-119]. Furthermore, data on children are scarce and recently performed studies have shown conflicting results [120, 121].

Thus, the aim of our studies in this thesis, paper III and IV, was to investigate the association of thyroid autoimmunity and thyroid function in 12-year-old children with celiac disease (treated and untreated) compared to their healthy peers (Paper III and IV).

Diagnosis of celiac disease

Serology: In the 1980s, sensitive and specific serological tests were developed for celiac disease and these are now used as a first triage. Serological testing of these celiac antibodies, IgA tissue transglutaminase (tTG), IgA endomysial (EMA), and IgA and IgG deamidated gliadin peptide antibodies (anti-DGP), has become more and more important for the diagnosis of celiac disease. The autoantibodies used for serologic testing of untreated celiac disease have among the highest sensitivity and specificity, approaching 95 %, compared to other autoantibodies for detecting autoimmune disorders [122]. All antibody-based serologic tests are expected to normalize on a gluten-free diet, thus, test accuracy depends on ongoing gluten consumption. This means that serological tests also can be used to monitor celiac disease activity.

One of the first antibodies to use was anti-gliadin antibody (AGA) during the 1980s. However, this test for both IgA and IgG has significantly lower sensitivity and specificity compared to the recently developed tests and the positive predictive value is less than 30%, thus, it is hardly used to find celiac disease anymore [122].

EMA (endomysial antibody) was developed in the middle of 1980s and has both a sensitivity and a specificity of above 90% in patients with overt villous atrophy. Unlike AGA, anti-tTG and –DGP assays, which are based on the enzyme-linked immunosorbent assay (ELISA), the EMA assay is based on immunofluorescence. It is significantly more expensive since it requires either monkey esophagus or human umbilical cord tissue as a substrate. There is also a high interobserver variability since it requires individual interpretation [122].

In 1997, tTG was identified as a celiac disease autoantigen [21], which resulted in the development of an ELISA-based test without the difficulties in immunofluorescence detection of patients' autoantibodies. ELISA tests are technically less demanding and less subjective than the indirect immunofluorescence methods used to detect EMA. TG-ELISA can be performed in standardized fashion in any clinical laboratory, and TG is the main EMA autoantigen. Accordingly, TG-ELISA is recommended as the primary serological screening test for celiac disease. The concordance rate between EMA and TG is very high and there is generally no need to utilize both [123].

The total IgA level should be measured before the test, because more than 2% of patients with celiac disease have a selective IgA deficiency. IgA autoantibody tests may be negative even in active celiac disease. In patients in whom low IgA or selective IgA deficiency is identified, IgG-based testing (IgG anti-DGP) and IgG tissue transglutaminase, anti-

tTG) should be performed [124]. Antibodies against DGP are based on the conversion of certain gluten peptides to deamidated peptides by the action of intestinal tTG. These peptides bind with high affinity to human leukocyte antigen DQ2 or DQ8 on celiac patients' antigen-presenting cells to potently stimulate the inflammatory T-cell response in the mucosa of patients with celiac disease. The result is an antibody response to these deamidated gliadin peptides that displays a higher specificity for celiac disease than antigliadin antibodies [37]. These antibodies are mostly used for identification of celiac disease in children below two years of age and in patients with IgA deficiency. The sensitivity and specificity of anti-DGP testing in this population seem to be equivalent to those of IgA anti-tTG [125, 126].

Histology: As described earlier, Paulley, 1954, reported that the clinical manifestations of celiac disease are linked to destruction of the lining of the small intestine. The subsequent development of the peroral intestinal biopsy 1955-1956, resulted in that the gluten-dependent enteropathy based on the histologic assessment of the intestinal mucosa became the standard for diagnosis of celiac disease [16]. Thus, for a long period, a small bowel biopsy was obtained by using a capsule. The capsule method is able to get biopsies from more distal parts of the upper small intestine and the specimens obtained are usually bigger in size compared to the samples obtained via endoscopy [14, 127]. The major problem is that only one biopsy can be obtained, hence, nowadays, most gastroenterological clinics use endoscopic biopsy using a small-diameter, flexible and fiberoptic instrument. This has many advantages, including that the mucosa of the upper gastrointestinal tract can be visualized and multiple biopsy samples can be obtained. The current recommendation is four-six specimens from different locations, including the duodenal bulb, since the bulb can be the only affected part [128]. Particularly four macroscopic endoscopic findings suggestive of villous atrophy have been described: loss of circular folds, mosaic pattern, scalloping and nodularity [129, 130], **Figure 4.**

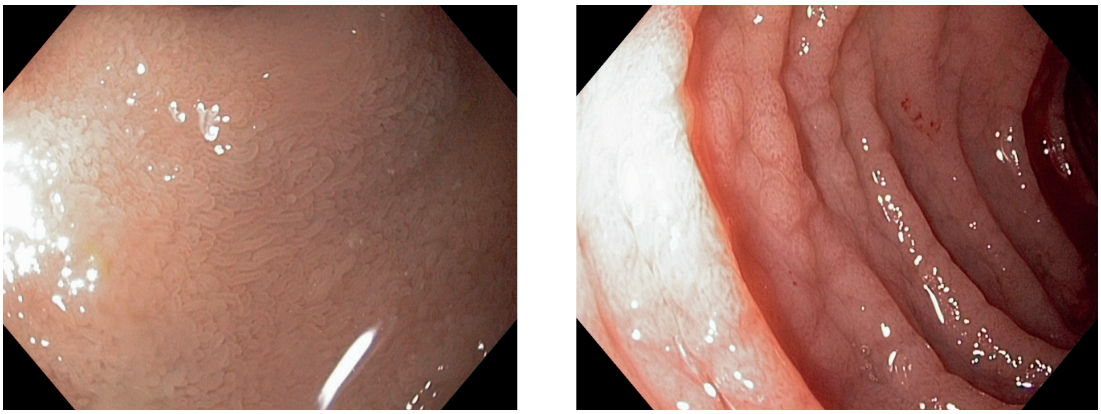


Figure 4. Left: Normal intestinal mucosa. Right: Mucosal damage due to celiac disease with scalloping and nodularity.

Microscopically, the mucosal changes are classified histologically according to Marsh [14-16]. The histological characteristics with villous atrophy and crypt hyperplasia and increased intraepithelial lymphocytes are hallmarks of celiac disease. The Marsh classification, modified by Oberhuber, is based on villous height and constitutes of four levels of intestinal damage. Marsh type 1 is increased number of lymphocytes on intact mucosa. Marsh type 2 and types 3a-c have increased levels of villous atrophy and crypt hyperplasia. Marsh type 4 is very rare and includes total atrophy of the mucosa including crypts [131]. It has to be noticed that the villous lesions can also be caused by for example infections such as Giardiasis, Helicobacter Pylori and also by immune deficiency, collagenous sprue and Crohn's disease. However, other diagnoses than celiac disease as causes of villous atrophy in Sweden are infrequent, **Figure 5**.

Genetic tests: Because individuals with celiac disease almost always have the DQ2 or DQ8 molecule, as described earlier, the absence of either of these molecules by genetic testing basically excludes the possibility of celiac disease. However, the presence of either molecule does not guarantee that celiac disease will develop in an individual. Thus, genotyping for the presence of HLA-DQ2/DQ8 has a high negative predictive value and is of value mainly for excluding the disease [89], **Figure 6.**

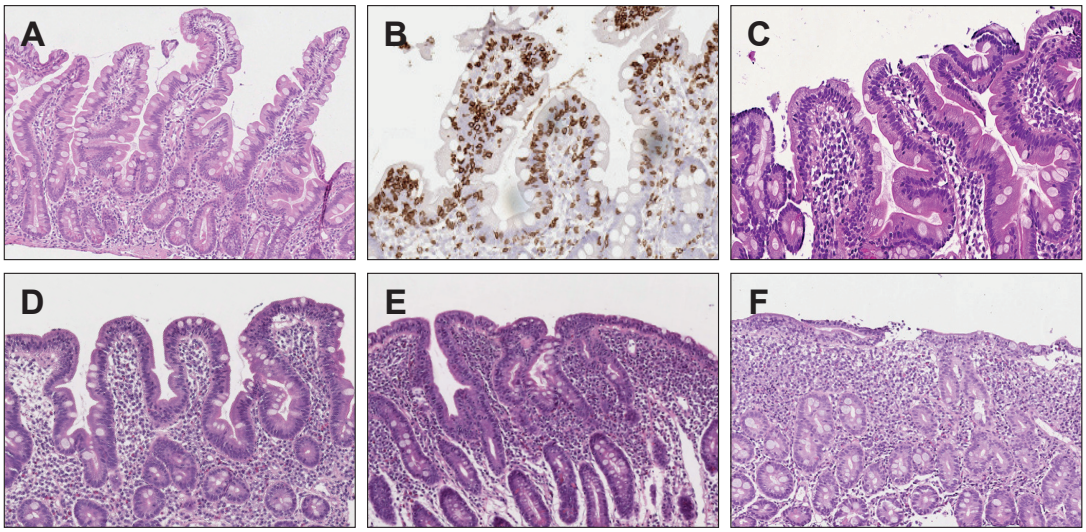


Figure 5. The most common histological mucosal changes classified according to Marsh, modified by Oberhuber. **A.** Normal mucosa **B.** Marsh type 1, intraepithelial lymphocytosis-positive immunostaining for CD3 **C.** Marsh type 1, intraepithelial lymphocytosis (IEL) **D.** Marsh type 3a, partial villous atrophy **E.** Marsh type 3b, subtotal villous atrophy **F.** Marsh type 3c, total villous atrophy. Images provided by Dr Patrick Joost, Department of Pathology, Skane University Hospital, Lund.

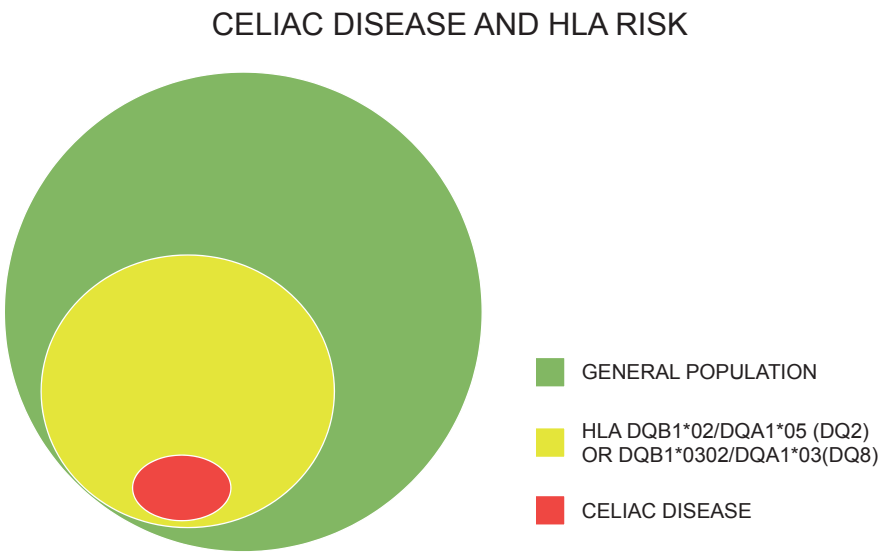


Figure 6. Figure showing the relationship between the general population, HLA-type and celiac disease.

Treatment

The only effective treatment today is a gluten-free diet, that is, a lifelong elimination of wheat, rye and barley from the diet. Non-dietary treatments are under investigation but still not used in clinical practice [132]. Although the nutritional diet is safe and effective, the implications of every day life and the social burden should not be neglected since grains are common staple food and, as described above, gluten is the base for bread, noodles, pasta, and other food products, widely used in the food industry [133]. Oats are considered relatively safe although it is not generally recommended since most of the commercially available oats are contaminated by gluten during growing, transportation and milling [1]. The elimination of gluten usually induces clinical improvement within days or weeks, though histologic recovery takes months or even years. Especially among adults, the mucosa may show a persistent abnormal histopathological appearance despite a clinical response [134].

Compliance with a gluten-free diet

A gluten-free diet can thus be challenging for the patient. A multidisciplinary approach is of uttermost importance to motivate the affected children and the role of the dietitian

is very important. Adherence to a gluten-free diet effectively results in a return to health [135]. A survey among teenage boys and girls with celiac disease showed that the nutrient intake of this age group with celiac disease was comparable to that of their healthy control peers [136]. Among the same group of children, both the children with screening-detected celiac disease and the ones with clinically diagnosed celiac disease still reported comparable health related quality of life as their peers without celiac disease [137]. However, many adolescents experience challenges related to dining services and social situations [138]. Another study show that patients with celiac disease are satisfied with the diagnosis and are motivated to adherence although particularly those who are diagnosed young, who suffer from extraintestinal symptoms or who are totally asymptomatic and diagnosed by screening require additional support [139].

Non-responsive celiac disease (NRCD)

This is defined as persistent symptoms, laboratory findings and signs of celiac disease despite at least 12 months of treatment with a gluten-free diet. The first step is to rule out other diagnoses since other causes to villous atrophy will require different treatments and do not respond to a gluten-free diet. A substantial proportion of patients are at risk of this condition, approximately between 7 and 30%. The condition is referred to as either primary if there is initial failure to respond to a gluten-free diet or secondary if symptoms or laboratory abnormalities consistent with celiac disease re-develop after an initial response to a gluten-free diet [140, 141]. Unintentional gluten exposure is the most common cause of NRCD. Other causes are irritable bowel syndrome, refractory celiac disease, lactose intolerance and microscopic colitis [141, 142].

A possible protective effect of a gluten-free diet

Studies have shown conflicting mortality data regarding undiagnosed celiac disease [143] although the majority of the studies have suggested that patients with undiagnosed, untreated, celiac disease have a slightly higher mortality rate than those with negative serology for celiac disease[144-146]. A large population-based study showed a modestly increased risk of death in both children and adults who were diagnosed with celiac disease, the risk decreasing with time, but still significant after five years of diagnosis[147]. Both patients with positive celiac disease antibodies but normal intestinal biopsy, ''latent celiac disease" and those with inflammation but without villous atrophy in duodenal biopsies were also at an increased risk of death. These patients had pathological changes that may be part of the spectrum of celiac disease but do not acquire the celiac disease diagnosis and are not traditionally prescribed a gluten-free diet [148]. Most of the

excess risk occurs in the first year after diagnosis suggestive that persisting inflammation is a contributing factor. Even if a patient is well-treated with a gluten-free diet, it takes time for the mucosa to heal. Cardiovascular disease and malignancy are among the main causes of death in celiac disease patients [147]. The mortality in celiac disease decreases over time with appropriate treatment [145, 146].

In the beginning of the 21th century, a few studies showed that an early diagnosis and subsequent treatment and adherence to a gluten-free diet could prevent the development of other autoimmune diseases and that the auto-antibodies for diabetes and thyroid disease among celiac disease patients tend to disappear on a gluten-free diet [114, 115]. However, the relationship between treating celiac disease and the prevention of autoimmune disease remains controversial and as described above, the role of gluten in associated autoimmune disorders is still unclear and other studies have shown contradictory results [117-119]. Among patients with type 1 diabetes and celiac disease, there is some evidence for a beneficial effect when adhering to a gluten-free diet, for example undetected celiac disease in these patients is associated with a higher prevalence of retinopathy, nephropathy and poor glycemic control [149, 150]. Notwithstanding, the evidence are not completely convincing [151]. There seems to be an increased prevalence of undiagnosed subclinical or silent forms of celiac disease in first-degree relatives of celiac disease patients as well as a high risk of autoimmune disorders. This indicates that for persons with a genetic predisposition for celiac disease, it is beneficial to be aware of not only the long-term risks of an unrecognized and untreated celiac disease as described above (i.e., malignancies, osteoporosis, infertility, and so on) but also for associated autoimmune conditions [95].

Refractory celiac disease

About 5% of the celiac disease patients are at risk of developing refractory sprue defined as persistent symptoms and lack of mucosal healing despite a strict gluten-free diet [152]. The symptoms include persistent nutritional deficiencies, weight loss, and malabsorption and it can be a precursor to enteropathy associated T cell lymphoma. Weight loss, a potential marker of celiac disease severity may be associated with lymphoma risk. Compliance to a gluten-free diet did not significantly alter the risk, but a moderate effect cannot be excluded [153, 154]. Refractory celiac disease patients who do not respond to a gluten-free diet and have aberrant intestinal T cells have greatly increased levels of homozygosity for the DR3-DQ2 haplotype (44–62%), compared with other celiac disease patients (20–24%) [27, 29, 32, 155].

Screening

If mass-screening for celiac disease or not should take place is still a matter of debate. The arguments against screening include low positive predictive values of current serological tests and that the natural course of celiac disease is still unknown. Other arguments against screening include difficulties in maintaining adherence to a gluten-free diet, conflicting mortality data regarding undiagnosed celiac disease as described above and lack of data regarding cost-effectiveness [156]. Advocates of a general screening note that celiac disease meets World Health Organization criteria for diseases that authorize mass screening: the condition is common; clinical detection is difficult; screening tests are highly sensitive and specific; effective treatment is available; and untreated disease can lead to complications [157]. The conflicting data with regard to mortality risk in undiagnosed celiac disease are likely due to differences in age, definitions of seropositivity, and follow-up time. However, the general perception is that arguments do not justify screening for celiac disease in the general population but instead focus on active case finding and screening in high-risk groups [156, 157].

Epidemiology and celiac disease in Sweden

The prevalence of celiac disease is 1 to 3% in the general population in Europe and North America and approximately 10% among first-degree family members of patients with celiac disease [1, 158, 159]. The prevalence has increased in developed countries over recent decades and there are large regional differences both between and within countries [160]. The occurrence of celiac disease resembles an iceberg since the majority of all cases remain undiagnosed [2, 5, 161].

Sweden experienced an "epidemic" of celiac disease (1984-1996) in children below two years of age [162], **Figure 7**. A four-fold increase in the incidence of clinically detected disease, followed by a comparable decrease one decade later, was confirmed through the National Swedish Childhood Celiac Disease Register [162-164]. The epidemic has been partly attributed to differences between the epidemic and post-epidemic birth cohorts regarding infant feeding, more specifically the proportion of infants breastfed at the time of gluten introduction and the amount of gluten fed to the infants during its introduction [162, 165]. In 1982, approximately two years prior to the beginning of the epidemic, Swedish pediatricians recommended postponing gluten introduction in infants from four until six months of age in an attempt to postpone the development of the disease, in accordance with the changed European recommendations at that time [166]. Later, it was also shown that the gluten content of Swedish industrially produced

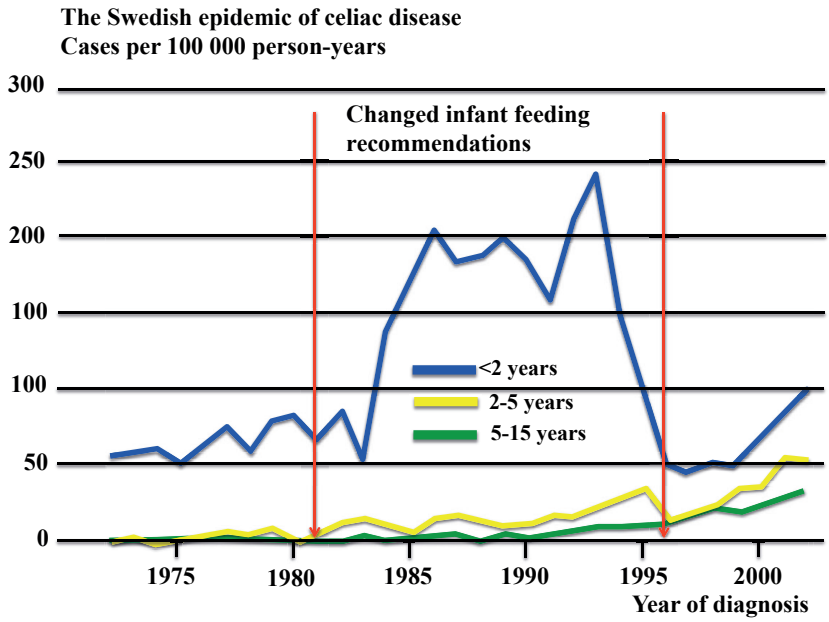


Figure 7. The Swedish epidemic of celiac disease with the arrows showing the point in time when the infant feeding recommendations changed thereby rendering the birth cohorts during and after the epidemic, exposed to different infant feeding [162, 164].

milk cereal-based follow-on formulas and porridges concurrently had been substantially increased in order to substitute for a reduction in milk, which was decreased to reduce the protein load in the infants [162]. During the epidemic when the incidence of celiac disease was high, a case referent study was performed, indicating a protective effect of breastfeeding and gluten introduction. Gluten introduced in small amounts during ongoing breastfeeding was preferable [165].

Taken together, the changes in infant feeding resulted in birth cohorts with different infant feeding patterns on a population level. During the epidemic, a more abrupt introduction to large amounts of gluten after weaning, was common and during the post epidemic period, introduction of small amounts of gluten during ongoing breastfeeding was the most common practice.

Whether infant feeding affects the occurrence of the disease, or merely the clinical expression and/or age at disease onset, is still unknown. Thus, the relationship between infant feeding and celiac disease risk, as well as between infant feeding and other autoimmune diseases and allergies, is still controversial, and evidence-based complementary feeding strategies are limited [73, 167].

Sweden has had an epidemic of celiac disease of the youngest children without counterpart in the rest of the world, which provides a unique opportunity to explore the causes of celiac disease and its consequences. As the Swedish epidemic of celiac disease was seen among clinically detected children it can be considered in two different ways: either as an increase followed by a decrease in the proportion of clinically detected cases (the children with symptoms) but without a change in the prevalence of the disease, or as a real change in celiac disease prevalence.

Aims

The overall aim was to investigate environmental factors related to the prevalence of celiac disease and to study associated clinical features among the affected children.

The specific aims were:

- To make an evaluation of the prevalence of celiac disease including both clinically- and screening-detected cases, in Swedish 12-year-old children born during the celiac disease epidemic and post-epidemic periods, and to relate these findings to changes over time in infant feeding (Paper I).

- To examine if weight and height can be used to predict celiac disease in children (Paper II).

- To assess any association between celiac disease, clinically detected (i.e. treated) and screening detected (i.e. untreated) and autoimmune diseases such as thyroid autoimmunity and thyroid dysfunction (Paper III and IV).

Subjects and methods

Overall study design

The ETICS study - Exploring the Iceberg of Celiacs in Sweden - was conducted in five study sites across Sweden: Umeå, Norrköping, Norrtälje, Växjö and Lund. The background to the study was the Swedish epidemic of celiac disease without counterpart in the rest of the world [162]. The study was designed by five pediatricians and later further developed by a multidisciplinary research group including the involved PhD students, research nurses, dietitians, health economists, epidemiologists and laboratory personnel, among others. The ETICS study is part of the 'Prevent CD' European project [168]. A two-phased celiac disease cross-sectional screening study was performed. The screening was school-based, performed in 2005–2006 and in 2009–2010, and included two birth cohorts of 12-year-olds, one representing the epidemic birth cohorts (born in 1993) and the other representing the postepidemic cohorts (born in 1997). The multicenter study covered the same geographic areas of Sweden, and each of the five sites included a major city with municipalities in the surrounding suburbs and countryside. Clinically detected celiac disease (i.e., celiac disease diagnosed within routine clinical care before the study) was reported at enrollment, but these children were also encouraged to take part in the study.

The **National Swedish Childhood Celiac Disease Register** was used to confirm the reported clinically detected celiac disease cases [163]. This is an incidence register, initiated in 1991 and in 1998, the register was expanded to become nationwide and to include all 47 pediatric departments. Reporting is based on a standardized form including parents' consent, personal identity number, gender, basis for diagnosis and date at diagnosis.

A total of 10041 children from the 1993 cohort were invited, with 7567 (75%) participating and 7208 (72%) blood sampled. Corresponding numbers for the 1997 cohort were: 8284 invited, with 5712 (69%) participating and 5424 (65%) blood sampled with the help of research nurses visiting the schools. In the 1993 cohort 8.8% of the population birth cohort was invited (cohort size: n=117997). The corresponding proportion for the 1997 cohort was 9.2% (cohort size: n=90502) (Statistics Sweden; www.scb.se). In total, 12632 children (69% of those invited) participated, with similar sex ratios in both birth cohorts, p=0.99 (48% and 49% girls in the 1993 and 1997 groups, respectively).

Screening strategy and definitions

Clinically detected celiac disease, was reported by the parents at enrollment and ascertained through the Celiac Disease Register and/or the child's medical record. The other children were screened through analysis of serological markers and all children with positive values were referred to the pediatric department at their study site for case ascertainment with small intestinal biopsy. Criteria for diagnosis were Marsh type 3a-c enteropathy, or the combination of Marsh type 1-2 enteropathy, HLA-DQ2/DQ8, symptoms or signs compatible with celiac disease, and clinical response to a gluten-free diet. Age at celiac disease diagnosis in clinically detected cases was defined as the age at the first small intestinal biopsy.

A summary of the screening strategy and case ascertainment, on which prevalence comparisons were based, resulting in a diagnosis of screening-detected celiac disease, i.e. celiac disease undiagnosed prior to the study and detected through the screening, is presented in **Figure 8.**

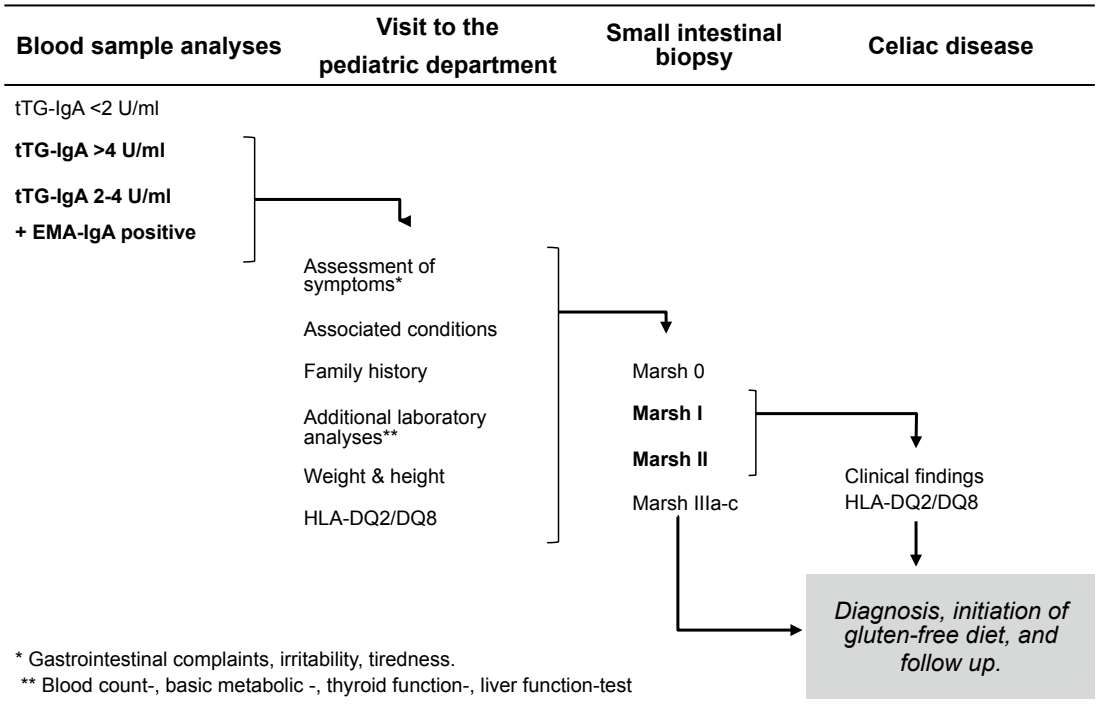


Figure 8. Figure depicting the screening procedure. Bold indicates further diagnostic procedures. The intestinal biopsies were blindly evaluated by a second pathologist. Adapted with permission from Anna Myleus' thesis, 2012.

Laboratory analyses to find celiac disease cases

All serum samples were analyzed for tTG-IgA and values above 4 U/mL were considered positive. Intermediate values of tTG-IgA (2-4 U/mL) were additionally analyzed for EMA-IgA with values equal to or more than a 1:5 dilution considered as positive. Children with tTG-IgA below 2 U/mL were classified as non-cases.

tTG-IgA was determined by conventional enzyme-linked immunosorbent assay using a commercial kit (Celikey®, Phadia GmbH, Freiburg, Germany). EMA-IgA was analyzed with indirect immunofluorescence technique using tissue sections from marmoset monkey esophagus mounted on glass slides (The Binding Site, Birmingham, UK). Sera yielding fluorescent binding to the endomysial structure were diluted to determine the lowest titer detectable. Analyses were performed according to the manufacturer´s instructions at the same laboratory.

While all samples in the first phase were analyzed for total serum-IgA, this was not performed in the second phase due to low yield (two additional cases found). IgA-deficiency was defined as serum levels below 0.06 g/l. When total serum-IgA was low (<0.5 g/l), analysis of tTG-IgG was performed. The cut-off for positive tTG-IgG was set at 6 U/ml, and serum samples with intermediate values (3-6 U/ml) were further analyzed for EMA-IgG with 1:5 dilution as the cut-off for positivity. Total serum-IgA levels were analyzed using a routine nephelometric method (BN Pro Spec® System, Dade Behring, Marburg GmbH, Germany). tTG-IgG and EMA-IgG were determined by the same methods as for the IgA analyses.

Prevalence comparisons were based on the same screening protocol in both phases, i.e. excluding the two cases found after analyses of IgG antibodies in the first phase.

Case ascertainment for screening-detected celiac disease cases

Children with values above the cut-off described above were referred to the closest pediatric department and recommended to undergo a small intestinal biopsy (**Figure 8**). Biopsies were taken mainly by gastroscopy with the recommended four to six biopsies from the duodenum and the bulb. In the first phase, a few clinics used a suction capsule but in the case of normal small intestinal mucosa, re-biopsy was performed using gastroscopy [169]. Histological assessment of the biopsies were performed according to the revised Marsh-Oberhuber classification [170]. A second pathologist blinded to the previous result, examined all the biopsies. In case of disagreement, a third pathologist evaluated the biopsy.

Children with screening-detected celiac disease were genotyped for HLA- DQ2/DQ8. DNA was extracted from whole blood using the Blood & Cell Culture DNA Kit (QIAGEN®, Hilden, Germany). Genotyping for HLA alleles encoding for DQ2/DQ8 was performed by multiplex-PCR reactions with oligonucleotide probe hybridization and detection on agarose gel (Eu-DQ® test, Eurhospital SpA, Trieste, Italy).

Comparison of changes in celiac disease prevalence over time and infant feeding (Paper I)

Paper I was based on the children from both cohorts, included in the ETICS study, described in detail above. The total number of screening-detected celiac disease cases and clinically detected cases for each birth cohort were compared and the changes in prevalence were related to infant feeding.

Ascertainment of infant feeding

Questionnaires regarding the differences in infant feeding between the cohorts were completed by 67% of the participating families before the results from the serological screening were known. The questions included breastfeeding duration and age at gluten introduction. The duration of breastfeeding was defined as the period of time when the infant was exclusively or partially breastfed. Age at introduction of gluten was the first month of life when flour from wheat, rye or barley was given. The background to the questionnaires was the changes in infant feeding demonstrated at the time of the Swedish epidemic as described earlier. Already known from previous studies were the differences in infant feeding between the cohorts summarized in **Table 1.** Infant

Table 1 - Characteristics of infant feeding in the two birth cohorts.

	Birth cohort of 1993	**Birth cohort of 1997**
Introduction of gluten-containing food		
From 6 months of age	X	
In small amounts, from 4-6 months of age		X
Average daily flour consumption[1] (g/child/day)	38	24
Proportion of infants breastfed[2]		
4 months of age (%)	77	83
6 months of age (%)	63	74

[1]In children below 2 years of age [162]
[2]Exclusively or partially breastfed. Statistically significant difference between cohorts at both ages (P<0.001) The National Board of Health and Welfare (www.socialstyrelsen.se)

feeding practices shifted from a favorable to an unfavorable pattern, and back again with respect to celiac disease risk, **Figure 7** [165, 171].

Growth parameters in the children with celiac disease compared to their healthy peers (Paper II)

Paper II was based on the children from both cohorts, included in the ETICS study, as described above. The total number of screening-detected celiac disease cases formed our celiac disease cohort. Children with an existing diagnosis of celiac disease (n = 96) were excluded from this study since the main objective was to examine the difference in growth parameters between untreated celiac disease cases and their healthy peers.

Anthropometric assessment

Weight and height were measured at the time of the screening for celiac disease according to standard procedures. All school nurses were given uniform instructions on how to carry out these measurements. The scales were recently calibrated, and a wall-mounted stadiometer was used for measuring height. The children wore light clothing and no shoes and were measured with their body in a straight line and their head in an appropriate position. BMI was calculated as weight (kg) divided by the square of the height (m^2) and categorized using the cut-off points recommended by the International Obesity Task Force (IOTF) [172]. Age- and sex- specific cut-off points corresponding to the adult BMI value of <18.5 (defined as underweight) and ≥ med 25 (defined as overweight) were used. As a reference, adult BMI 25 corresponds to 21.22 for 12-year-old boys and to 21.68 for 12-year-old girls and adult BMI 18.5 corresponds to 15.35 for 12-year-old boys and to 15.62 for 12-year-old girls [172, 173].

A nested case-control design within the ETICS study on thyroid autoimmunity and thyroid function (Paper III and IV)

Among the 12632 children who had their blood sampled in the ETICS study described above (69% of those invited), we identified 335 celiac disease cases (200 girls) whereof 93 had previously diagnosed celiac disease and 242 (139 girls) were screening-detected within the ETICS-study**.**

A nested case-control design was used to evaluate the risk of thyroid autoimmunity related to the treated, i.e. the previously diagnosed celiac disease cases and untreated screening-detected celiac disease cases, respectively. The main analyses of papers III and IV were hence based on 335 celiac disease cases as described above and 1695 controls, matched for sex and age. The controls were randomly selected from all cohort members free of celiac disease at the time of diagnosis, with a final ratio of 5 controls per case. The mean age at diagnosis among the previously diagnosed celiac disease cases was 4.7 and the median was 2.8 years. At 12 years of age (i.e. the age of the screening-detected and the controls when the screening was performed), the previously diagnosed cases had thus been on a gluten-free diet approximately 8 years. The majority of the previously detected (83/93) had a normal tTG at the time of the screening and the rest had a slightly raised tTG (range 4.1 to 29.9). The total number of celiac disease cases formed our celiac disease case group. This group was used as a basis for screening of thyroid autoimmunity and thyroid function.

Thyroid autoimmunity and thyroid function

Autoantibodies of IgG type directed against TPO were measured in blood samples and used as an indicator of thyroid autoimmunity and expressed as arbitrary units per milliliter (U/mL). The cut-off value for TPO positivity was set to 100 U/mL (Varelisa TPO Antibodies, Phadia GmbH, Freiburg, Germany). TSH and fT4, used as markers of thyroid function, were determined with reagents from Roche (Roche Diagnostics, Mannheim, Germany) on Cobas e602. The TSH assay is standardized against the 2nd International Reference Preparation (IRP) WHO Reference Standard 80/558. The limit of detection and functional sensitivity of the TSH assay are 0.005 and 0.014 mIU/L, respectively. Reference range (normal) for the test was 0.51-4.3 mIE/L. The standardization of the fT4 assay is traceable to an equilibrium dialysis method. The fT4 assay has a measuring range of 0.3-100 pmol/L. Reference range (normal) for the test was 12-22 pmol/L.

Thyroid function was classified using the American Thyroid Association guidelines [174]. Overt hypothyroidism was demonstrated by an increase in TSH serum concentration above the reference range described above and a significant decrease in fT4 serum levels below the reference range. Subclinical hypothyroidism was noted by an increase in TSH serum concentration and normal fT4. Overt hyperthyroidism was noted by a decrease in TSH serum levels below the reference range and an increase of fT4 above the reference range; subclinical hyperthyroidism was indicated by a decrease of TSH serum levels and normal fT4.

Statistical Analyses

Power of the ETICS study and estimation of sample size

The same total celiac disease prevalence in the two birth cohorts was our prespecified null hypothesis. The acceptable limit for a Type I error was set at 5% (α=0.05), and for a Type II error at 10% (β=0.10), corresponding to a statistical power (1-β) of 90%. Considering an assumed difference in prevalence between the cohorts of 0.5%, it was necessary to have a sample size of approximately 7 000 children, with approximately 5 000 consenting to participate, from each cohort.

The same statistical significance was set in all four studies, defined as an odds ratio (OR) with a confidence interval (CI) not including 1, or a two-tailed P≤0.05. Microsoft Access 2010 (Microsoft, Redmond, WA) was used for handling the ETICS database, and statistical analysis was performed using SPSS Statistics for Windows (Version 21.0, IBM Corp, Armonk, NY).

Paper I

Prevalence was reported as cases per 1000 individuals with a 95% confidence interval (CI) and percentages. Prevalence comparisons between cohorts were calculated by using the log-transformation method (Open Access program WinPepi 11.8) [175] and expressed as prevalence ratios with a 95% CI and P values. Comparisons for proportions and medians were performed by using the chi-square test and the Mann-Whitney U test, respectively.

Paper II

Continuous variables were reported as the median and interquartile range (IQR) because of unequal sample size and skewed distributions. The 25th and 75th percentiles are described in the text. Categorical variables were reported as the number and percentage of subjects with the characteristic of interest. Between-group comparisons were performed with the Mann-Whitney U test for continuous variables and with chi-square or Fisher's exact test for categorical variables as appropriate. These tests were performed on the whole group as well as after stratifications. Univariate logistic regression models tested the odds of having celiac disease while being underweight or being overweight.

Paper III and IV

The relation between celiac disease and TPO Abs positivity and between TPO Abs positivity and hypothyroidism were analyzed with logistic regression. Results were presented as odds ratios (OR) with 95% confidence intervals (CI) and the 95% CI not including 1.0 were considered statistically significant.

Ethical considerations

The Regional Ethical Review Board of Umeå University, Umeå, Sweden, approved the ETICS study [Dnr UmU 04-156M]. All studies complied with Swedish legislation (e.g., the Personal Data Act; SFS 1998:204 and the Biobanks in Medical Care Act; SFS 2002:297) and the Helsinki declaration. The established personal register within the study was listed at Umeå University [dnr UmU 101-2496-04]. Written informed consent was obtained from the parents or legal guardians of all children participating in the screening.

Results

Prevalence of childhood celiac disease and changes in infant feeding (Paper I)

Comparison between birth cohorts

The screening procedure revealed a total celiac disease prevalence of 29 in 1000 in the 1993 cohort, including both clinically and screening-detected cases [3] and 22 in 1000 in the 1997 cohort. A significantly lower risk for celiac disease in children born after the celiac disease epidemic (1997) compared with during the epidemic (1993) was observed at 12 years of age (prevalence ratio: 0.75; 95% CI: 0.60–0.93; P = 0.01; **Figure 9**). Comparison between birth cohorts of clinically detected cases resulted in a prevalence ratio of 0.68 (95% CI: 0.45–1.0; P = 0.07), showing a trend towards significance. We found a significantly lower prevalence of positive serological markers in the 1997 cohort compared with the 1993 cohort (prevalence ratio: 0.74; 95% CI:

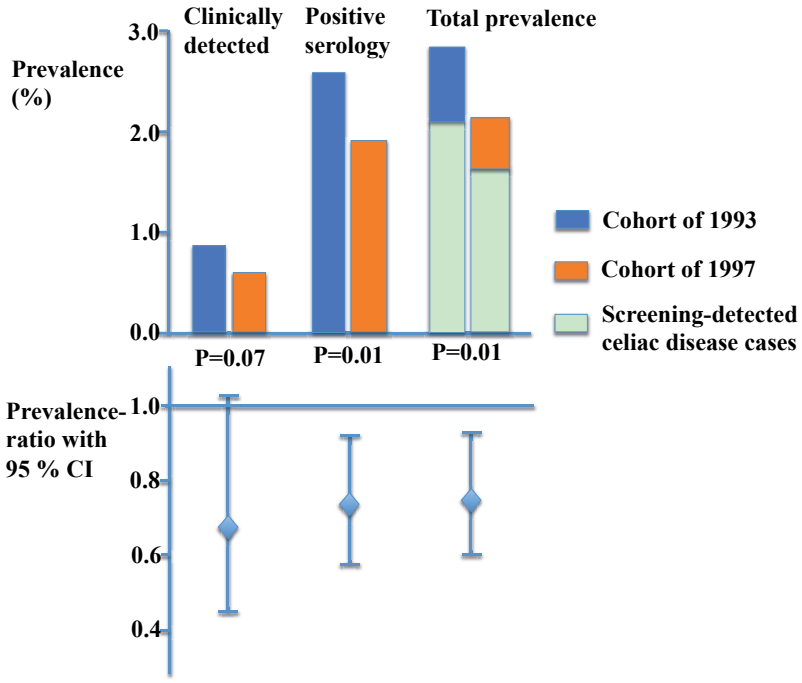


Figure 9 - Comparison of the two cohorts. A statistically significant difference in the celiac disease prevalence is shown.

0.58–0.94; P = 0.01), and the difference remained in biopsy-verified cases (prevalence ratio: 0.78; 95% CI: 0.6–1.0; P = 0.06), approaching statistical significance (**Figure 9**). There was no difference between the birth cohorts in the proportion of clinically vs. screening-detected cases (30% vs. 28% clinically detected cases; P = 0.59). Among the clinically detected cases, there was a significantly lower median age at diagnosis for the birth cohort of 1993 than the cohort of 1997 (1.7 vs. 5.5 years; P = 0.04). Considering both cohorts together, celiac disease was more common among girls than boys (prevalence ratio: 1.6; 95% CI: 1.3–2.0; P < 0.001), but the difference was more pronounced in the 1997 cohort (prevalence ratio: 1.8 vs. 1.4).

Screening of the 1993 Cohort

The main findings are summarized in **Table 2** and **Figure 10**. Clinically detected celiac disease was identified in 66 cases, all but one had their diagnosis confirmed with a duodenal biopsy. The non-biopsied case had EMA 1/320, family history of celiac disease, and clinical response to a gluten-free diet. Positive tTG-IgA and enteropathy were found in 151 screening-detected cases, all carrying the HLA-DQ2/DQ8 haplotype (**Table 2**).

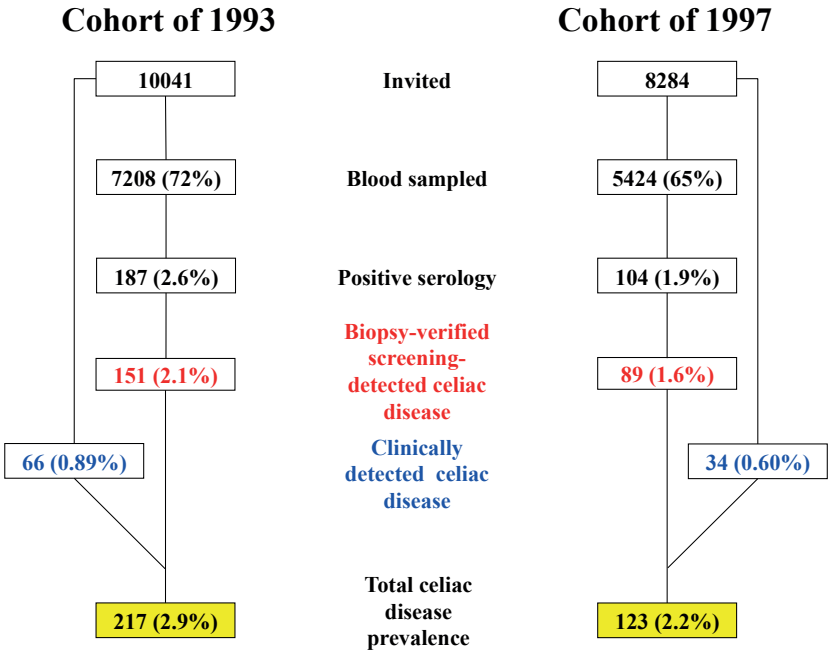


Figure 10 - Cross-sectional screenings of celiac disease in two birth cohorts. Numbers are given in the boxes with the percentage shown in brackets.

Table 2 - Celiac disease screening outcome with respect to serological markers, biopsies and HLA genotyping in two birth cohorts of 12-year-olds.

	1993 Cohort		1997 Cohort	
	n (%)	(%)	n	(%)
Positive serological markers				
tTG-IgA >4 U/mL	167	(89)	85	(82)
tTG-IgA 2–4 U/mL and EMA-IgA >1:5	20	(11)	19	(18)
Total	187		104	
Girls	100		68	
Small intestinal biopsy evaluation				
Marsh type 3	139	(92)	88	(99)
Marsh type 2	2	(1.3)	0	
Marsh type 1 and symptoms/signs[1]	9	(6.0)	1	(1.0)
Non-interpretable	1	(0.7)[2]	0	
Total number of cases	151		89	
Girls	80		57	
HLA genotyping				
DQ2	113	(75)	71	(80)
DQ2/DQ8	23	(15)	8	(9.0)
DQ8	15	(9.9)	9	(10)
Non-DQ2/DQ8	0		0	
Not available	0		1	(1.0)[3]

[1] Symptoms included gastrointestinal complaints, deviation in weight or height, anemia, fatigue, heredity for celiac disease and other autoimmune disorders. [2] Diagnosis based on tTG>100 after gluten-challenge, biopsy non-interpretable and parents declined repiobsy. [3] Diagnosis based on tTG >100 U/mL and Marsh type 3 enteropathy

Screening of the 1997 Cohort

Clinically detected celiac disease, confirmed with a duodenal biopsy, was identified in 34 cases, of which 22 were girls and 12 were boys, corresponding to a prevalence of 6.0 in 1000 (95% CI: 4.1–8.3; **Figure 10**). Positive serological markers were found in 104 children, corresponding to a prevalence of 19 in 1000 (95% CI: 16–23). Small intestinal biopsies and mucosal evaluation were performed in 99 children (95%). Five families declined additional investigation. We identified 89 children who fulfilled the diagnostic criteria (**Table 2**). The prevalence of biopsy-verified screening-detected celiac disease was 16 in 1000 (95% CI: 13– 20). The total celiac disease prevalence in the 1997 cohort was 22 in 1000 (95% CI: 18–26; Fig 9), with a prevalence of 28 in 1000 (95% CI: 22–35) among girls and a prevalence of 15 in 1000 (95% CI: 11–20) among boys. All successfully genotyped screening-detected cases (99%) carried the HLA-DQ2/DQ8 haplotype (**Table 2**).

Infant Feeding in the Study Population

Duration of breastfeeding was seven and nine months in the 1993 and 1997 cohort, respectively ($P < 0.001$), which is in agreement with the proportion of infants breastfed at six months of age in the Swedish population (**Table 2**). Median age at gluten introduction was five months in both cohorts; nevertheless, the proportion of infants with breastfeeding continuing beyond gluten introduction was significantly larger in the later cohort (70% vs. 78% in the 1993 and 1997 cohort, respectively, $P < 0.001$). Comparable infant feeding patterns were observed for the celiac disease cases and the respective study population in each cohort (data not shown).

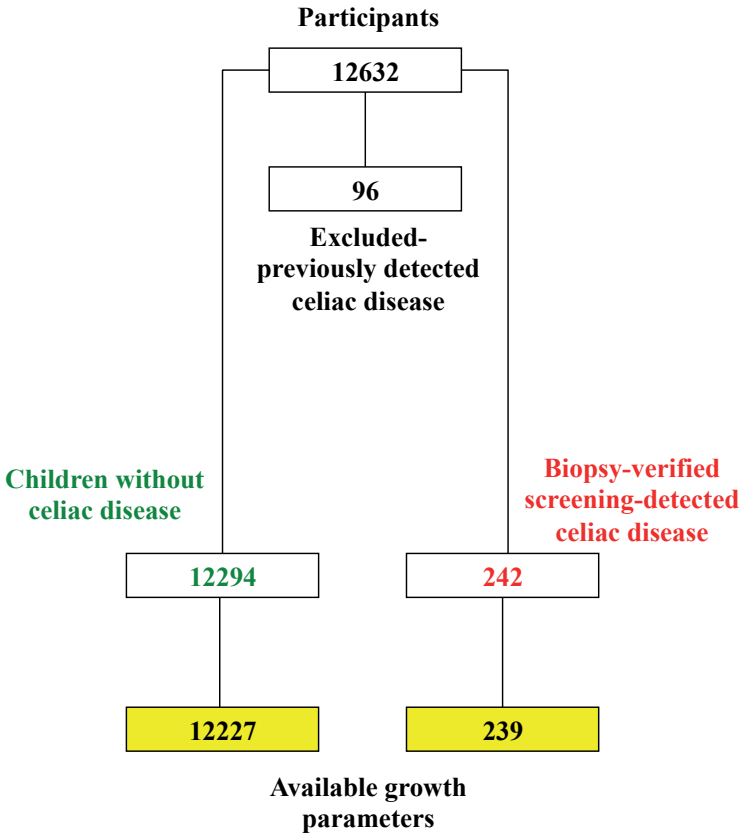


Figure 11 - Flowchart depicting the screening procedure. Cross-sectional screenings performed in 12-year-old children across Sweden to investigate the prevalence of celiac disease and to assess the growth parameters in comparison with healthy children. The numbers of children are given in the boxes.

Body mass index is not a reliable tool in predicting celiac disease in children (Paper II)

In total, 12632 children (69% of those invited) participated as described earlier. Out of these, 242 newly detected celiac disease cases were identified within the study as a result of the screening. Weight and height were available in 239 (99%) children with newly detected celiac disease (57.3% girls) and in 12227 (99%) of the study participants without celiac disease (48.5% girls) (**Figure 11**).

The children with screening-detected celiac disease weighed on average less compared to the children without celiac disease (median weight 45.2 kg, IQR 41.1– 54.4 kg vs. 47.0 kg, IQR 40.2–52.2 kg, p = 0.01) (**Table 3**). There was no statistically significant difference in weight between girls and boys within the celiac disease group (p = 0.86). The children with screening-detected celiac disease were also significantly shorter compared to the children without celiac disease (median height 156.5 cm, IQR 151.0–162.0 cm vs. 157.5 cm, IQR 152.0–163.0 cm, p = 0.04). The distribution of underweight, normal weight, and overweight differed significantly between the groups. Among the patients with screening-detected celiac disease, 4.2% were underweight, 82.0% were of normal weight, and 13.8% were overweight. In the group of healthy children, the proportions were 5.2%, 72.8% and 21.9%, respectively (**Table 3 and Figure 12**). Using children with normal weight as a reference group, there was no association between

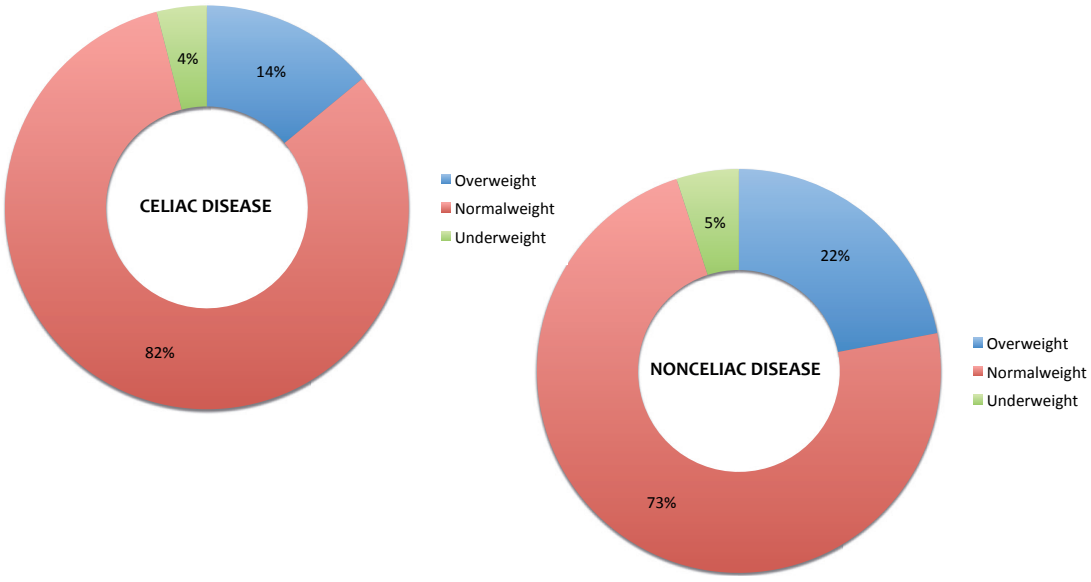


Figure 12. The distribution between overweight, normal-weight and underweight differed between the celiac disease patients and their healthy peers.

Table 3 - Comparison of BMI, weight and height between children without celiac disease (CD) and children with screening-detected celiac disease.

GROUPS, n (%)	No CD n=12227	CD n=239	P-value[a]
Girls, n (%)	5932(48.5)	137(57.3)	
Age-and sex-adjusted BMI, n (%)			
<18.5 kg/m^2	641(5.2)	10(4.2)	
18.5-24.9 kg/m^2	8906(72.8)	196(82.0)	
≥25 kg/m^2	2680 (21.9)	33(13.8)	0.01*
BMI (kg/m^2) median (IQR)			
All	18.8(3.85)	18.6(3.15)	0.05*
Girls	18.8(3.84)	18.6(3.37)	0.22
Boys	18.8(3.88)	18.6(2.67)	0.13
Weight (kg) median (IQR)			
All	47.0(13.3)	45.2(12.0)	0.01*
Girls	47.1(12.9)	44.9(13.0)	0.07
Boys	47.0(13.6)	46.2(11.0)	0.09
Height (cm) median (IQR)			
All	157.5(11.0)	156.5(11.0)	0.04*
Girls	158.0(9.9)	156.0(10.4)	0.04*
Boys	157.0(11.2)	157.0(12.6)	0.32

[a] Statistical significance defined as P≤0.05 and marked with *

being underweight and the risk of having undiagnosed celiac disease (OR 1.3, 95% CI 0.7–2.4). However, the risk of having celiac disease was significantly lower among overweight children (OR 0.56, 95% CI 0.4–0.8).

BMI was slightly lower among the children with screening-detected celiac disease compared to their healthy peers (median 18.6 kg/m^2, IQR 17.1–19.8 kg/m^2 vs. 18.8 kg/m^2, IQR 17.2–21.1 kg/m^2, p = 0.05).

Among the girls with screening-detected celiac disease (n = 137), 2.2% were underweight, 83.9% were of normal weight, and 13.9% were overweight compared to 6.6%, 73.8%, and 19.8% of their healthy peers, respectively (p = 0.02). The girls with screening-detected celiac disease were also significantly shorter compared to their healthy peers (median height 156.0 cm, IQR 151.6–162.0 cm vs. 158.0 cm, IQR 153.0–162.9 cm, p = 0.039). Among the boys with screening-detected celiac disease (n = 102), 6.9% were underweight, 79.4% were of normal weight, and 13.7% were overweight compared

Table 4 - Comparison of BMI between girls and boys without celiac disease (CD) and girls and boys with screening-detected celiac disease.

GROUPS, n (%)	No CD	CD	p-value
Age- and sex-adjusted BMI			
Girls	5932(48.5)	137(57.3)	
<18.5 kg/m^2	391(6.6)	3(2.2)	
18.5-24.9 kg/m^2	4376(73.8)	115(83.9)	
≥25 kg/m^2	1165(19.6)	19(13.9)	0.02*
Boys	6295(51.5)	102(42.7)	
<18.5 kg/m^2	250(4.0)	7(6.9)	
18.5-24.9 kg/m^2	4530(72.0)	81(79.4)	
≥25 kg/m^2	1515(24.0)	14(13.7)	0.03*

[a] Statistical significance defined as P≤0.05 and marked with *

to 4.0%, 72.0%, and 24.1%, respectively, among their healthy peers (p = 0.03). Overall, the distribution of underweight, normal weight, and overweight in sex-stratified subgroups showed a similar pattern as the whole group (**Table 4**).

Thyroid autoimmunity and thyroid function in children with celiac disease compared to healthy 12-year-olds (Paper III and IV)

Thyroid autoimmunity

TPO status was obtained in 2023 (99.7%) out of the 2030 children in our nested case-referent study. A higher proportion of the children with celiac disease than their healthy peers showed signs of thyroid autoimmunity, defined as high titers of TPO Abs (17/242 (7.0%) (OR 2.6, 95% CI 1.5-4.6) vs. 7/93 (7.5%) (OR 2.8, 95% CI 1.2-6.4) vs. 48/1695 (2.8%), screening detected vs. previously detected vs. healthy. Thus, the proportion having positive TPO Abs was equally high in both celiac disease groups.

Thyroid function

TSH, fT4 and TPO status for determination of thyroid disease classes were obtained in 1949 (96.1%) out of the 2030 patients. In 80 patients (3.9%) insufficient blood sample volume precluded laboratory analysis. Among the 72 patients with TPO positivity, TSH status was obtained in 70 children.

Table 5. Thyroid involvement and thyroid disease classification of the study participants *with and without* thyroid autoimmunity, respectively.

Thyroid function in the TPO Abs-positive group[a]	**No CD n=46 (%)**	**Screening CD n=14 (%)**	**Previously CD n=7 (%)**
Overt hypothyroidism	1 (2.2)	2 (14.3)	1 (14.3)
Subclinical hypothyroidism	11(23.9)	5 (35.7)	2 (28.6)
Euthyroidism	34 (73.9)	7 (50.0)	2 (28.6)
Subclinical hyperthyroidism	---	---	2 (28.6)
Overt hyperthyroidism	---	---	---
Thyroid function in the TPO Abs-negative group[b]	**No CD n=1585 (%)**	**Screening CD n=202 (%)**	**Previously CD n=82 (%)**
Overt hypothyroidism	---	---	---
Subclinical hypothyroidism	99 (6.2)	18 (8.9)	6 (7.3)
Euthyroidism	1484 (93.6)	184 (91.1)	75 (91.5)
Subclinical hyperthyroidism	1 (0.1)	---	1 (1.2)
Overt hyperthyroidism	1 (0.1)	---	---

[a] Three children could not be classified according to the American Thyroid Association guidelines and were excluded in the table above. [b] Ten children could not be classified according to the American Thyroid Association guidelines and were excluded in the table above.

Of those children with TPO positivity, 18 (25.7%) had subclinical hypothyroidism and four (5.7%) had overt hypothyroidism. Nobody had overt hyperthyroidism but two (2.8%) showed signs of subclinical hyperthyroidism. Among children without TPO Abs, 123 (6.3%) showed subclinical hypothyroidism and no one had overt hypothyroidism whereas two (0.1%) had subclinical hyperthyroidism and one (0.1%) had overt hyperthyroidism. Detailed information describing the thyroid disease classes in each celiac disease group and among their healthy peers, with and without signs of thyroid autoimmunity respectively is shown in **table 5**. Overall the group with TPO Abs positivity (with or without CD) showed an odds ratio of 7.2 (95% CI 4.2–12.2, $p < 0.001$) for hypothyroidism.

Hypothyroidism was more common in children with celiac disease and TPO Abs positivity than in children without celiac disease but with TPO Abs (10/21 (47.6%) vs. 12/46 (26.1%), thus an increased risk for children with celiac disease + TPO Abs compared to healthy children + TPO Abs (OR 3.1, 95% CI 1.03–9.6, $p = 0.044$).

In all groups, the OR of developing hypothyroidism with a positive TPO Abs titer was: (a) 5.3 (CI 2.7-11.0) in healthy 12-year olds; (b) 10 (CI 3.2-32.0) in screening-detected

Table 6 - The Odds Ratio of having thyroid dysfunction in the different celiac disease groups using healthy children as a reference.

Groups	n	OR	95 % CI	p-value
Healthy	1631	1.0	---	
Previously detected CD	89	2.1	1.1-4.0	0.023
Screening detected CD	216	1.8	1.1-2.8	0.016
All CD	305	1.9	1.3-2.7	0.002

Abbreviations: OR=odds ration; CI=confidence interval CD=celiac disease

celiac disease cases; (c) 19 (CI 2.6-135.0) in previously detected celiac disease cases; (d) 12 (CI 4.4-32.0) in the celiac disease case group. In general, (without consideration to TPO Abs status), having celiac disease almost doubled the risk of having thyroid dysfunction **(Table 6)**.

TPO Abs-negative children with celiac disease did not have an increased risk of hypothyroidism compared to their healthy peers without TPO Abs (24/284 (8.4%) vs. (99/1585 (6.2%), all CD vs. healthy, OR 1.4, 95% CI 0.87-2.2, p = 0.17).

Discussion

Methodological considerations

Research designs

Randomized controlled trials are often considered the gold standard in evidence-based medicine and optimal for e.g. pharmaceutical trials. However, many research questions cannot be approached by such a design due to practical and ethical constraints, and in addition the generalizability of the results to the real world can be limited. On the other hand, observational study designs involve individuals in their natural environment both with respect to exposures (e.g. infant feeding) and outcomes (e.g. celiac disease), taking advantage of the variation in exposures between individuals and sometimes also changes in exposure over time [177, 178].

The key difference between the two designs is that in the randomized controlled trial the exposure is by purpose and randomly allocated to different individuals (as an experiment), while the observational designs take advantage of variation in exposure between individual that occurs outside the control of the researcher. The latter implies that potential confounding needs to be taken care of in the stage of analyses instead. Irrespective of study design - experimental or observational – each step of the study needs to be scrutinized with respect to quality [179].

Cross-sectional studies: A cross-sectional study is an observational study in which exposure and outcome are determined simultaneously for each subject [180]. In this research project, the celiac disease screening of the adolescents had a population based cross-sectional design, considered preferable for determining the prevalence. The primary limitation of the cross-sectional study design is that because the exposure and outcome are simultaneously assessed, there is generally no evidence of a temporal relationship between exposure and outcome. Consequently, as we aimed to discern whether the prevalence of celiac disease (including both clinically detected and screening-detected cases, the latter previously unrecognized) had changed over time, we conducted two large cross-sectional screening studies consecutively with four years apart and otherwise with a methodology that was as similar as possible. Including all the children in the same age group added more strength to the study. As it previously had been shown that infant feeding had changed over time [162, 171, 181] this implies that we approached

two birth cohorts with different exposures, but with the same follow-up time for both (i.e. up to 12 years of age). Thus, our design could be described as quasi-experimental taking advantage of changes that has been imposed on the population from outside, but not by the researchers.

Cohort studies: The identifying feature of a cohort study design is that the subjects are followed over time. Cohort studies begin with all individuals of a certain group, thus contains individuals that are exposed and not exposed to a factor of interest, respectively, and then evaluate the subsequent development of outcomes in both groups [182]. When a prospective cohort design is used, these studies are typically time consuming and expensive, although historical cohort studies for example based on registers bypass these challenges. Both a cross-sectional study and a cohort study can be used as the sampling frame for a **nested case-control study** as in paper III and IV in this research project. The cases and controls are both chosen from a defined group and in this project from the two cohorts of children born 1993 and 1997. By utilizing data previously collected from our screening study, the time and cost of beginning a new case-control study is avoided. The case control design is often used when the exposure of interest is difficult or expensive to obtain and when the outcome is rare [179].

Potential errors in observational studies

Sources of errors in epidemiology can be *random* or *systematic*. Before concluding that an individual study's conclusions are valid, one must consider the following sources of error that might provide an alternative explanation for the findings.

An example of **random error** is sampling error and the best way to reduce this is to increase sample size. Sample size in turn affects precision [179, 182]. Sample size must be large enough to reach statistical significance if there is an existing difference between the compared groups. In this project, we had enough statistical power to determine the difference in prevalence of celiac disease in the different cohorts, paper I (see under Method Section). In the case-control study (paper III and IV) the number of cases was pre-determined, however, the statistical power was increased by selecting more than one control per case and by a procedure of matching for certain characteristics, as age and sex. The sample size was large enough to be convincing with respect to the variables studied although the statistical power was not sufficient enough to detect smaller effects or to perform stratified analyses. The precision of the study was improved by an appropriate ratio of matched controls selected for each case. Whereas precision refers

to lack of random error, validity refers to a lack of systematic error [182]. We will first describe systematic error before mentioning validity.

In contrast to random error, **bias** refers to **systematic errors** in any type of epidemiologic study that result in an incorrect estimate of the association between exposures and outcomes [179].

Selection bias appears when there is a systematic differences between the people included in the study and those not participating in the study. In both study cohorts, we invited nearly 10% of the total Swedish birth cohort, and the study sites were selected to represent the country from north to south. Participation rate was high in both cohorts with only marginal differences between the sites. The populations in the sites had similar consumption of health resources per capita and unemployment rates as the national population (www.scb.se). For these reasons, we consider the samples as likely to be representative of the country. We also consider the children from the birth cohort 1993 representative for the birth cohorts from the decade of the epidemic period. The participating students' ethnic origin was considered representative of the different ethnicity in Sweden as the ETICS study was school-based. Thus, it did not affect the proportion of invited children with a specific genetic predisposition. Preliminary results of genotyping for HLA-DQ2/DQ8 in a random sample in the 1993 and 1997 cohorts, respectively, suggest that the proportion of children predisposed to celiac disease was somewhat larger in the 1997 cohort, thus not explaining the lower prevalence seen in this cohort.

Sample selection in a case-control study is complex. The most important issue to consider is the process by which controls were selected and the resulting comparability of cases and controls. The controls in our studies were different compared to the cases with respect to the celiac disease diagnosis, but matched for age and sex to increase similarity and thereby handling some aspects of potential confounding.

Measurement bias occurs when the individual measurements or classifications of disease or exposure are inaccurate and ***recall bias,*** a differential recall of information by cases and controls, is one of the most important to consider here [179]. Recall bias can be reported either in a baseline questionnaire as in our screening studies, or in a case control study where subjects with disease may remember past exposures differently than those who do not have the disease. In the screening studies, we used questionnaires to collect infant feeding data about breastfeeding duration and age at gluten introduction.

The questionnaires were completed before knowing the screening results, thus, even if this was a cross-sectional design, this part can be considered as prospective, minimizing recall bias. The recall time was 12 years, and the parents might have difficulties in remembering infant feeding data. However, the parents in both cohorts are likely to remember equally poorly and non-differential misclassification might underestimate a potential difference. Difficulties with remembering age at gluten introduction were reflected by 13% missing answers in otherwise completed questionnaires. We have previously shown that approximately 70% of the parents during the epidemic followed the infant feeding recommendations and another study have shown that 60% of the parents to children born after the epidemic [64] followed the revised recommendation. Overall, there could have been a difference in age at gluten introduction but it might not be evident after 12 years.

Our screening strategy with serological markers prioritized high sensitivity in order to identify as many potential cases as possible, but in order to avoid non-cases misclassified as cases, a small intestinal biopsy evaluating the mucosa and the presence of HLA-DQ2/DQ8 were required for celiac disease diagnosis. To reduce the number of children undergoing an unnecessary small intestinal biopsy (false positive markers) we used two serological markers in sequence for some of the children; first tTG with a lowered cut-off to increase sensitivity, and then EMA for all children with intermediate values. The mucosal evaluation was also blinded for two and in some cases where the mucosa was difficult to classify, even three different pathologists. The screening procedure was identical in both the cohort of children born 1993 and in the children born 1997.

We used criteria for celiac disease that also included minor enteropathy (Marsh type 1 and type 2). However, restricting the diagnostic criteria to villous atrophy (Marsh type 3), as proposed in the recent European guidelines [89] did not alter the conclusion (prevalence ratio, 0.79; 95% CI:0.63–0.98; P = 0.03).

In the BMI study, the weight and height measurements were performed according to standard procedures (as opposed to self-reported), and this makes them very reliable and comparable, thus minimizing measurement bias.

Confounding comes from the Latin word ‘confundere’ meaning ‘to mix together’ and is a major challenge in epidemiological research [179, 182]. For a variable to be a confounder it must be a risk factor for the outcome on its own and it may create a cause-effect relationship that is not true. Confounding by age and gender in this project was dealt with by matching, as performed in our nested case-control study. There are

several methods in the analytical phase to handle confounding, such as stratification and multivariate methods.

Validity means that a test measures what it is intended to measure, that is, there should be no systematic error and the random error should be as small as possible [179]. Observational studies are evaluated in terms of both internal and external validity. *External validity* is the ability to generalize study results to a more universal population and internal validity is a prerequisite for external validity. We believe that our large birth cohorts in the cross sectional screening studies are highly representative for the 12-year-old children in the country in general. The *internal validity* of a study is defined as the extent to which the observed difference in outcomes between the two comparison groups can be attributed to the intervention rather than other factors. By minimizing the bias as described above, we believe that the studies in this thesis all have high validity.

Findings and implications

The **main finding** in this thesis is a surprisingly high total celiac disease prevalence among 12-year-olds in Sweden and the majority of the cases were unrecognized prior to the screening in both cohorts. The Swedish celiac disease epidemic was observed among clinically detected cases below two years of age. We have now shown that this difference in clinically detected disease remained at 12 years of age. The prevalence among children born 1997 was 2.2%, thus, a statistically significant decrease in total prevalence compared to the children born 1993, during the epidemic, where the prevalence of celiac disease was 2.9%. The finding of a difference in total prevalence (ratio 0.75; 95% CI, 0.60-0.93, figure 10) in two similar birth cohorts four years apart leads us to believe that celiac disease, to some extent, can be prevented by changing environmental- and/or lifestyle factors. The main difference between the cohorts was the infant feeding practices, as shown by the population data ascertained through questionnaires, and our findings suggest that introduction of gluten-containing foods during ongoing breastfeeding is favorable with respect to celiac disease risk. With the exception of infant feeding, no other major changes in environmental- or lifestyle factors in Swedish society were found during this time period.

Moreover, we found that, at a population level, children with celiac disease weigh less, are shorter, and have a lower BMI compared to their peers without celiac disease, and this emphasizes the importance of early recognition and treatment of the condition. However, at an individual level, growth parameters are not reliable in predicting celiac disease. Nonetheless, celiac disease has been associated with several other diseases,

increased morbidity, and moderately increased mortality and diagnosis and treatment are regarded as beneficial [147, 183-185].

The association with other autoimmune conditions, among them insulin dependent diabetes mellitus and thyroid diseases such as Hashimoto thyroiditis, are well known [102-104]. However, the role of gluten in associated autoimmune disorders is still unclear and other studies have shown contradictory results [117-119]. We found the prevalence of thyroid autoimmunity among 12-year-old children with celiac disease to be almost three times higher than in the age- and sex- matched control group of children without celiac disease. We did not find any difference in the prevalence of thyroid autoantibodies in the group of children with previously diagnosed (treated) celiac disease compared to the screening-detected (untreated) cases. Having celiac disease in addition to TPO Abs, increased the risk of thyroid dysfunction, mainly presented as subclinical hypothyroidism. Below are the individual papers discussed separately.

Prevalence of celiac disease and changes in infant feeding (Paper I)

Our screening verifies that the epidemic pattern in celiac disease incidence seen in Sweden between 1984 and 1996 represents a change in the actual disease occurrence and not only a change in clinical presentation [162, 164]. Previous studies have indicated that infant feeding affects the clinical presentation and/or age at disease onset [186, 187]. In line with these findings, we found a higher median age at diagnosis among the clinically detected cases born after the epidemic compared to those born before.

Infant feeding recommendations The beginning and the end of the Swedish epidemic of celiac disease were preceded by changes in infant feeding recommendations and more specifically, regarding the age for introduction of gluten-containing complementary food. The changes were implemented through the well-baby clinics that are attended by almost all infants (99%, www.socialstyrelsen.se). In 1982, the recommendation was changed to introduce gluten-containing food at six months of age instead of four, and in 1996 the recommendation was revised again, now recommending that gluten could be introduced from four months of age. From the questionnaires completed before awareness of the screening results, no difference in age at gluten introduction could be observed. Nonetheless, we found a difference between the cohorts in the proportion of infants being introduced to gluten during ongoing breastfeeding, probably a combined effect of the revised infant feeding recommendation and the

increase in duration of breastfeeding (**Table 1**). Contemporaneously, but independently of the revised feeding recommendations, the gluten content of Swedish milk and cereal–based follow-on formulas was first substantially increased and later decreased again [162]. The current Swedish infant feeding recommendation, revised as a result of the celiac disease epidemic, implies both the aspects of gluten introduction in small amounts and concomitant breastfeeding. Our findings suggest that this is favorable with respect to celiac disease risk. In both European and US infant populations, similar infant feeding practices have been recommended [188-190].

The hypothesis that early exposures influence celiac disease development is supported by our previous pilot screening study, performed on the same birth cohorts, suggesting a difference in celiac disease prevalence as early as 2.5 years of age [191] Oral tolerance to an antigen develops early in life, and celiac disease can be viewed as a failure to develop oral tolerance to gluten, or a later loss of this tolerance. The development of oral tolerance is a complex immunologic process involving interactions between genetic factors and environmental and lifestyle exposures, such as bacterial gut colonization and infant feeding [73, 192].

Breastfeeding Breastfeeding has been associated with reduced risk for several autoimmune diseases and for allergy [189]. With respect to celiac disease, we have previously shown a protective effect of concomitant breastfeeding and introduction of gluten-containing complementary foods [165], an effect also seen in other studies but not in all [69, 193]. The current study suggests that this protective effect prevails up to 12 years of age. Introducing gluten during ongoing breastfeeding may increase the chance of developing oral tolerance through immune-modulating factors in breast milk and/or influence on the gut maturation and colonization [192]. Differences in the microbiota composition between formula-fed and breastfed infants have been shown, which might involve an increased celiac disease risk because differences in gut microbiota between individuals with and without celiac disease have been reported [194, 195]. Furthermore, breastfeeding has been associated with a reduced risk of gastrointestinal infections, an additional risk factor for celiac disease [62, 63, 189].

Age at gluten introduction As mentioned above, there might be a certain age interval that provides a window of opportunity with respect to developing oral tolerance [73, 192]. This window has been suggested to occur between four and six months of age [70]. However, evidence concerning the most favorable age to introduce gluten is still inconclusive [193, 196, 197]. Whether age between four and six months is preferable

for gluten introduction, in contrast to gluten avoidance until 12 months of age, has been under investigation in a prospective intervention study. The primary outcome was the prevalence of celiac disease autoimmunity and overt celiac disease among the children at five years of age. Neither the delayed introduction of gluten nor breast-feeding was found to modify the risk of celiac disease among at-risk infants, although the later introduction of gluten was associated with a delayed onset of disease. A high-risk HLA genotype was an important predictor of disease [198]. Another intervention study had the primary outcome to study the frequency of biopsy-confirmed celiac disease at three years of age after 475 participants had received 100 mg of immunologically active gluten daily, and 469 received placebo from 16 to 24 weeks of age. Breast-feeding, regardless of whether it was exclusive or whether it was ongoing during gluten introduction, did not significantly influence the development of celiac disease or the effect of the intervention. As compared with placebo, the introduction of small quantities of gluten at 16 to 24 weeks of age did not reduce the risk of celiac disease by three years of age in this group of high-risk children [199]. The general opinion is that introduction of large amounts of gluten should be discouraged. Earlier data has shown that exposure to gluten before four months of age increases the risk for celiac disease autoimmunity and is not recommended [188]. The interventional studies show that early introduction of gluten in genetically at-risk children may lead to earlier development of celiac disease autoimmunity and celiac disease even if it does not seem to be preventable [198]. The early occurrence of celiac disease may have an adverse effect on children's growth and nutritional status.

We have previously shown an increased celiac disease risk associated with gluten consumption in larger compared with smaller amounts two weeks after its introduction [165], and this is supported by our current findings because the cohorts differed in the amount of gluten ingested during weaning (**Table 1**). In agreement, a correlation between national celiac disease prevalence and wheat consumption has been shown [29], and celiac disease cases seem to react to gluten ingestion in a dose-response fashion [200, 201].

There are a few important differences in the intervention studies described above and our studies. First, our studies are observational which means that we perform a direct observation of individuals in their natural environment, exposed to 'real' gluten in contrast to the gluten in the intervention study. Also, we studied children at an intermediate risk for celiac disease while both the intervention studies focused on high-risk children. In the intervention studies, there was also a mixture of genetic risks with different HLA types and no assessment of non-HLA genes. Other environmental

factors except from gluten consumption, age at introduction and breastfeeding were not controlled for.

In our study, the majority of the participants did not have a relative with celiac disease. Thus, one can hypothesize that the participants in the intervention studies are already at high risk and have such a strong autoimmune predisposition where breastfeeding is not protective as opposed to the 'intermediate risk' participants in our studies. It is shown that the genotype of infants at family risk of developing celiac disease, carrying the HLA-DQ2 haplotypes, influences the early gut microbiota composition. Furthermore, it is suggested that a specific disease-biased host genotype may also select for the first gut colonizers and could contribute to determining celiac disease risk [202]. The participants in the intervention studies had one first-degree relative with celiac disease and it is not specified whether it is the mother or the father. The feeding patterns between children to mothers with celiac disease do not seem to vary compared with the offspring to those without celiac disease [203]. However, a breastfeeding child where the nursing mother has celiac disease and is currently on a gluten free diet is not exposed to gluten via the breast milk. These children have been shown to have a different microbiota compared to breastfeeding children to mothers who are on a regular diet [204]. Moreover, the same study showed that breast-feeding reduced the genotype-related differences in microbiota composition, which could partly explain the protective role attributed to breast milk in celiac disease [204]. Maybe breastfeeding and the gradual introduction of gluten are protective against celiac disease only in the group of children, carrying an intermediate risk but not in the high-risk group? In our study, the cohorts differed in infant feeding, as shown on a population level and ascertained through questionnaires. Because the infant feeding exposure pattern changed in the whole population, the proportion of children with the proposed worst combination (large amounts of gluten after discontinued breastfeeding) decreased, which could be associated with the reduced prevalence in the 1997 cohort. Although this so called "population experiment" was not intentional, it bears resemblance to an intervention study, and the relationship between infant feeding and celiac disease risk can thus be evaluated on a population level.

One disadvantage of our observational design is that the observed associations do not allow us to establish causality, and confounding factors can only be partially reflected on. Thus, our conclusion can be incorrect. However, to the best of our knowledge, there has not been any other major change in lifestyle- or environmental- factors during this time period, with the exception of infant feeding. Other explanations for the difference in the results between our study and the intervention trials could be factors such as

genetic susceptibility or environmental aspects specific for our country. For example, compared to many other countries, Sweden has a higher prevalence of celiac disease in children in general. Often, disparities in findings between the Swedish and non-Swedish populations are being observed [3, 34].

BMI and celiac disease (Paper II)

Children with untreated celiac disease were moderately shorter, weighed less, and had a slightly lower BMI compared to their healthy peers. Even though having undiagnosed celiac disease was not associated with being underweight, only a few were overweight and the majority of the children with screening-detected celiac disease had a normal weight. Our findings are in line with other studies. In 2004, an American study found that children with tTG-positive screening-identified celiac disease weighed less compared to healthy children and other recent studies have found that children with celiac disease are less frequently overweight or obese and more often underweight than controls [91, 93]. Some studies in patients with celiac disease have also found other factors associated with low BMI such as the extent of mucosal injury, presentation with diarrhea and female sex [79]. Studies performed in adults have shown similar results as in this study with regard to BMI [77, 205]. Even if celiac disease is classically associated with malabsorption and weight loss, one must bear in mind that children with undiagnosed celiac disease can have different body compositions. The fact that the proportion of underweight children with celiac disease in our study was only 4.2% emphasizes that celiac disease should not be considered primarily as a malabsorption disorder. In addition, although celiac disease was found in this study to be distinctly less common among overweight children, being overweight does not necessarily rule out celiac disease.

Gender differences In some studies, overweight has been found to be more common in boys with celiac disease than in girls [79, 206]. In contrast, in the present study, the same weight pattern was found to apply to both boys and girls and no indications of any gender differences in the proportion of overweight within the celiac disease group were found. In the current study, girls with celiac disease were significantly shorter than their healthy peers. This is consistent with other studies, including one study conducted in Finland where the screening-detected adolescent celiac disease subjects were not only shorter but one third also had nutritional abnormalities [92, 207]. Even more extensive nutritional deficiencies were found in a recently conducted Dutch study in which 7.5% of the individuals with celiac disease were found to be underweight but

the nutritional deficiencies were present even in obese patients [208]. This is important to keep in mind when considering the need for more active case finding of celiac disease in order to prevent the progression of nutritional deficiencies.

Height The results regarding height are more inconsistent than those for weight. In some studies, adult men with celiac disease have been found to be shorter [205, 209], but another study indicated that the mean adult height of patients with celiac disease was the same as that of the general population. In a subgroup analysis, reduced height was observed in the older, but not younger, birth cohorts with celiac disease [210]. Some other studies found that celiac disease diagnosed in childhood results in catch-up growth once a gluten-free diet is introduced, but still others found that men and women with celiac disease were shorter compared to controls [211-213]. Part of the differences found regarding height in the above-mentioned studies might be explained by differences in the investigated age span in the study populations. In the present study, it is likely that a majority of the girls at 12 years of age have reached the onset of puberty and that they are in the middle of their growth spurt. The onset of puberty corresponds to a biological (i.e., skeletal) age of approximately 11 years in girls and 13 years in boys. The timing of the pubertal growth spurt occurs earlier in girls and tends not to reach the same magnitude as that of boys. Girls average a peak growth velocity of 9 cm/year at age 12 and a total gain in height of 25 cm during the pubertal growth period. Boys attain an average peak growth velocity of 10.3 cm/year – which occurs about 2 years later than in girls – and gain 28 cm in height during the pubertal growth period [214-216]. This means that the majority of the girls participating in this study were in the middle of their peak height velocity when the study took place. It is possible, therefore, that untreated celiac disease gives them worse preconditions for the growth spurt or that the disease influences the onset of puberty and results in a delay in peak growth velocity. Additional epidemiologic research is needed to confirm these results, and more research needs to be done to understand the pathogenesis of short stature in patients with celiac disease.

Thyroid autoimmunity and thyroid function in children with celiac disease compared to their healthy peers (Paper III and IV)

We found the prevalence of thyroid autoimmunity among 12-year-old children with celiac disease to be almost three times higher than in the age- and sex- matched control group of children without celiac disease. Having celiac disease in addition to TPO Abs increases the risk of thyroid dysfunction among 12-year-olds. The most prevalent manifestation of thyroid dysfunction when having celiac disease in addition to TPO Abs was hypothyroidism. The majority of the children presented with subclinical hypothyroidism. The proportion of children having hypothyroidism was substantially higher in the celiac disease case groups compared to the children with thyroid autoimmunity but without celiac disease (OR 3.1). In children with celiac disease but without TPO Abs, there was no increased risk of having hypothyroidism compared to their healthy peers. Accordingly, the increased prevalence of TPO Abs among children with celiac disease, seems to be an important factor in explaining why thyroid dysfunction is twice as common in children with celiac disease compared to their healthy peers.

Gluten Treatment with a gluten-free diet did not appear to change the course of the autoimmune affection since there was no difference in the prevalence of TPO Abs among treated and untreated celiac disease patients [176]. The group of previously detected celiac disease patients in our study had been treated for several years with a gluten-free diet, on average 8 years. Adherence to a gluten-free diet among children is generally good as was adherence in the current study [176, 217-219]. Nevertheless, many celiac patients develop autoimmune disorders despite strict adherence to a gluten-free diet. The results are in accordance with other studies. The period of gluten exposure does not seem to be of compelling importance regarding development of autoimmune diseases [96, 117]. In fact, in one of the studies, the patients developed other autoimmune diseases to a lesser extent the longer the gluten exposure [96]. Conceivably, these patients, who were diagnosed with celiac disease at a later age, might be generally less prone to develop autoimmune diseases. This is also supported by another study where it was shown that a late diagnosis of celiac disease was associated with a decreased risk of autoimmunity. The same researchers found that the risk of development of an autoimmune disease in celiac patients was increased in patients with a family history of autoimmune diseases when celiac disease was diagnosed early in childhood or adolescence compared to adulthood [217]. A recent meta-analysis found that about 6%

of individuals with type 1 diabetes had celiac disease [220]. The association of celiac disease and type 1 diabetes is partly explained by shared HLA genes. Loci within the HLA region are also shared with thyroid autoimmunity. It supports the theory about a linkage disequilibrium between the genes responsible for celiac disease and those responsible for the co-expressed autoimmune diseases [221]. Moreover, above 60% of celiac disease-associated loci outside the HLA region identified by GWAS studies are shared with at least one other autoimmune disease [222]. This is in line with our findings and advocates a common genetic susceptibility to the autoimmune conditions.

Contrary to the above-mentioned findings, Ventura et al showed that patients with celiac disease had a high prevalence of both insulin dependent diabetes mellitus autoantibodies and thyroid-related serum autoantibodies. These autoantibodies were supposed to be gluten-dependent, since they disappeared during treatment with a gluten-free diet [115]. They also observed that the prevalence of autoimmune disorders in children in whom celiac disease was diagnosed before the age of two years, and who were treated with a gluten-free diet, was comparable to that of controls [114]. In our study, the distribution of TPO positivity at 12 years of age among the previously diagnosed celiac disease cases was equal among those diagnosed before two years of age and after two years of age. It was also equally distributed regardless of the degree of mucosal damage in the entire celiac disease cohort.

TPO Antibodies TPO Abs are indicators of autoimmune susceptibility. The clinical significance of these antibodies in celiac disease is still not clear but our results are consistent with previous findings that there is a higher propensity overall for both children and adults with positive TPO markers to develop autoimmune thyroiditis, defined as TPO Abs-positivity in combination with goiter and/or hypothyroidism [223]. In the present study, having TPO Abs regardless of celiac disease status increased the risk of having signs of hypothyroidism (OR 7.2). No one without TPO Abs had overt hypothyroidism. Thus, having TPO Abs substantially increases the risk of becoming hypothyroid and that risk is even higher if you also have celiac disease. This is consistent with a recently conducted Italian study where the researchers found that in children with Hashimoto's thyroiditis, having celiac disease, elevated TSH and TPO Abs at presentation were some of the predictive factors for thyroid failure in these patients [120]. Similar results have been shown in both adults and children with celiac disease [118, 224].

Hypothyroidism The majority of the children in our study with signs of hypothyroidism had subclinical disease. Among studies in adults, it has been shown that the presence of TPO Abs in serum is an independent risk factor for the development of overt hypothyroidism in patients with subclinical hypothyroidism [112, 225]. The Whickham study demonstrated that the presence of antithyroid microsomal (TPO is the antigen involved in the 'microsomal' response) antibodies, or elevated serum TSH alone, was associated with a significant increased risk of developing hypothyroidism at 20 years [112]. The same study also showed that women with thyroid autoantibodies (11% of the population) had an eight-fold higher likelihood of developing overt hypothyroidism over 20 years than did antibody-negative women. In women with both thyroid autoantibodies and isolated thyrotropin elevation, the risk of progression to overt hypothyroidism was 38 times higher with a 4% annual risk of developing overt hypothyroidism. On the contrary, studies in children indicate that autoimmune thyroiditis in children and adolescents seem to show a very low cytotoxic activity with a tendency to develop overt hypothyroidism only in few of the cases and it did not seem to worsen the situation if the children had celiac disease in addition to thyroid disease [223, 226]. The rate of progression to overt hypothyroidism ranged between 0% and 12.8% during the follow-up period in three studies [223, 227, 228]. Nonetheless, the researchers above could not find reliable prognostic factors to predict disease evolution in children and they therefore suggested that a regular screening for thyroid function should be performed in all patients with celiac disease who have positive antithyroid antibody titers. Altogether, it appears likely that subclinical hypothyroidism in children is a benign and remitting process with low risk of progression toward overt hypothyroidism. A longitudinal follow-up study is warranted in order to address the clinical significance of having subclinical hypothyroidism in 12-year-olds and in particular in children with subclinical hypothyroidism in addition to celiac disease. In conclusion, children with celiac disease, even if well treated, have a higher prevalence of thyroid autoimmunity than their healthy peers. Having TPO Abs in addition to celiac disease increases the risk of thyroid dysfunction, mainly expressed as subclinical hypothyroidism.

Concluding remarks and future perspectives

We have used different epidemiological designs to gain knowledge about a disease that is becoming a major public health issue. Through celiac disease screening (paper I), we found the highest celiac disease prevalence (2.9%) reported in Europe or the US in a birth cohort of the epidemic period (1993). Furthermore, we found a lower but still unexpectedly high prevalence (2.2%) in a birth cohort of the post-epidemic period (1997). In comparison with findings in previous studies, this prevalence indicates that celiac disease has increased over time in Sweden. When comparing the prevalence of these two cohorts we found a statistically significant difference in total celiac disease prevalence, including both clinically- and screening-detected cases. Thus, it seems as if celiac disease can be prevented in some cases, at least up to 12 years of age. We believe that the differences in celiac disease prevalence can be explained by changes in infant feeding during the period. Our findings suggest that gradual introduction of gluten-containing foods, preferably during ongoing breastfeeding, is favorable. Studies are warranted to further distinguish between duration of breastfeeding, gluten introduction with respect to age and amount and the overlap between breastfeeding and gluten introduction. The recently published randomized controlled trials regarding gluten introduction and breastfeeding among high-risk children are very interesting [198, 199]. Similar population based intervention studies among children with intermediate risk for celiac disease would be highly appreciated.

The majority of the children with screening-detected celiac disease were of normal weight and there was no association between being underweight and the risk of having undiagnosed celiac disease (paper II). At a population level, the 12-year-old children with screening-detected celiac disease weighed less and were shorter compared to their peers without celiac disease, and this indicates a need to detect and treat celiac disease. However, at the individual level growth parameters are not reliable in predicting celiac disease. Although celiac disease is less common in the overweight subgroup, being overweight does not rule out celiac disease. The growth parameters, and thus the BMI, of the children were available only at the time of the screening. Repeated weight and height measurements following the initiation of a gluten-free diet would permit an evaluation of the effect of the diet on the nutritional status of the children and would be interesting to do as a follow-up study.

Children with celiac disease are at an increased risk of other autoimmune conditions and in this thesis we have focused on thyroid disorders (paper III and IV). In conclusion, children with celiac disease, even if well treated, have a higher prevalence of thyroid autoimmunity than their healthy peers. Having TPO Abs in addition to celiac disease increase the risk of thyroid dysfunction, mainly expressed as subclinical hypothyroidism. It seems reasonable to conclude that measuring TPO Abs in euthyroid subjects in specific risk groups, such as celiac disease patients, can be used to identify subjects with increased risk for hypothyroidism. TPO Abs can be used as a first triage: screening by TSH measurement could be done only in the TPO Abs-positive subjects in a certain group. We did not find any difference in thyroid autoimmunity among the treated and untreated celiac disease cases but further research is warranted to evaluate gluten ingestion and its role in autoimmunity in general. Our results favor a common genetic susceptibility to the conditions. One of the most striking observations from GWAS results is this overlap in signals between different diseases [229] and it would be of particular interest to find out more about the connection between different autoimmune diseases.

Screening for thyroid disorders among celiac disease patients is briefly mentioned above and people with celiac disease have an increased risk of autoimmune disorders as compared with the general population. However, the relationship between associated autoimmune disease and celiac disease goes in both directions and for example, among those who have a family history of celiac disease, among those with Down syndrome, Turner's syndrome, or type 1 diabetes, there is an increased prevalence of celiac disease [1]. There is also an increased risk of non-celiac autoimmune disease in first-degree relatives of celiac disease patients [98]. Accordingly, it is highly recommendable to be aware of celiac disease as well and to test for the disease at wide indications. Serologic tests for celiac disease are easily available and provide accurate information about celiac disease status. As described above, celiac disease is a very heterogeneous disorder with many associated autoimmune conditions and inflammatory disorders. Future possible complications and what it means to have potential celiac disease (positive serology but a normal intestinal biopsy) still have to be ruled out [140]. The general perception is that arguments do not justify screening for celiac disease in the population. However, screening in high-risk groups may have a better cost-effectiveness and in such populations, such as those mentioned above, the higher prevalence of celiac disease will result in fewer false-positive serologic results [156, 157].

References

1. Green PH, Cellier C: **Celiac disease**. *The New England journal of medicine* 2007, **357**(17):1731-1743.
2. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, Murray L, Metzger MH, Gasparin M, Bravi E *et al*: **The prevalence of celiac disease in Europe: results of a centralized, international mass screening project**. *Annals of medicine* 2010, **42**(8):587-595.
3. Myleus A, Ivarsson A, Webb C, Danielsson L, Hernell O, Hogberg L, Karlsson E, Lagerqvist C, Norstrom F, Rosen A *et al*: **Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic**. *Journal of pediatric gastroenterology and nutrition* 2009, **49**(2):170-176.
4. Green PH, Jabri B: **Coeliac disease**. *Lancet* 2003, **362**(9381):383-391.
5. Lionetti E, Catassi C: **New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment**. *International reviews of immunology* 2011, **30**(4):219-231.
6. Zawahir S, Safta A, Fasano A: **Pediatric celiac disease**. *Current opinion in pediatrics* 2009, **21**(5):655-660.
7. S. G: **On the coeliac affection.** *St Bart Hosp Rep* 1890(24):17-20.
8. Losowsky MS: **A history of coeliac disease**. *Dig Dis* 2008, **26**(2):112-120.
9. Dowd B, Walker-Smith J: **Samuel Gee, Aretaeus, and the coeliac affection**. *British medical journal* 1974, **2**(5909):45-47.
10. Tommasini A, Not T, Ventura A: **Ages of celiac disease: from changing environment to improved diagnostics**. *World journal of gastroenterology : WJG* 2011, **17**(32):3665-3671.
11. van Berge-Henegouwen GP, Mulder CJ: **Pioneer in the gluten free diet: Willem-Karel Dicke 1905-1962, over 50 years of gluten free diet**. *Gut* 1993, **34**(11):1473-1475.
12. Anderson CM, French JM, Sammons HG, Frazer AC, Gerrard JW, Smellie JM: **Coeliac disease; gastrointestinal studies and the effect of dietary wheat flour**. *Lancet* 1952, **1**(6713):836-842.
13. Dicke WK, Weijers HA, Van De Kamer JH: **Coeliac disease. II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease**. *Acta Paediatr* 1953, **42**(1):34-42.
14. Crosby WH, Kugler HW: **Intraluminal biopsy of the small intestine; the intestinal biopsy capsule**. *The American journal of digestive diseases* 1957, **2**(5):236-241.
15. Paulley JW: **Observation on the aetiology of idiopathic steatorrhoea; jejunal and lymph-node biopsies**. *British medical journal* 1954, **2**(4900):1318-1321.
16. Marsh MN: **Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue')**. *Gastroenterology* 1992, **102**(1):330-354.

17. Kivel RM, Kearns DH, Liebowitz D: **Significance of Antibodies to Dietary Proteins in the Serums of Patients with Nontropical Sprue**. *The New England journal of medicine* 1964, **271**:769-772.

18. Seah PP, Fry L, Rossiter MA, Hoffbrand AV, Holborow EJ: **Anti-reticulin antibodies in childhood coeliac disease**. *Lancet* 1971, **2**(7726):681-682.

19. Maki M, Holm K, Lipsanen V, Hallstrom O, Viander M, Collin P, Savilahti E, Koskimies S: **Serological markers and HLA genes among healthy first-degree relatives of patients with coeliac disease**. *Lancet* 1991, **338**(8779):1350-1353.

20. Chorzelski TP, Sulej J, Tchorzewska H, Jablonska S, Beutner EH, Kumar V: **IgA class endomysium antibodies in dermatitis herpetiformis and coeliac disease**. *Annals of the New York Academy of Sciences* 1983, **420**:325-334.

21. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D: **Identification of tissue transglutaminase as the autoantigen of celiac disease**. *Nature medicine* 1997, **3**(7):797-801.

22. Molberg O, McAdam SN, Korner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P *et al*: **Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease**. *Nature medicine* 1998, **4**(6):713-717.

23. Catassi C, Fabiani E, Ratsch IM, Coppa GV, Giorgi PL, Pierdomenico R, Alessandrini S, Iwanejko G, Domenici R, Mei E *et al*: **The coeliac iceberg in Italy. A multicentre antigliadin antibodies screening for coeliac disease in school-age subjects**. *Acta Paediatr Suppl* 1996, **412**:29-35.

24. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E: **Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer**. *The Journal of experimental medicine* 1989, **169**(1):345-350.

25. Louka AS, Sollid LM: **HLA in coeliac disease: unravelling the complex genetics of a complex disorder**. *Tissue antigens* 2003, **61**(2):105-117.

26. Sollid LM, Thorsby E: **HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis**. *Gastroenterology* 1993, **105**(3):910-922.

27. Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, Ciclitira PJ, Sollid LM, Partanen J: **HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease**. *Human immunology* 2003, **64**(4):469-477.

28. Kupfer SS, Jabri B: **Pathophysiology of celiac disease**. *Gastrointestinal endoscopy clinics of North America* 2012, **22**(4):639-660.

29. Abadie V, Sollid LM, Barreiro LB, Jabri B: **Integration of genetic and immunological insights into a model of celiac disease pathogenesis**. *Annual review of immunology* 2011, **29**:493-525.

30. Megiorni F, Mora B, Bonamico M, Barbato M, Nenna R, Maiella G, Lulli P, Mazzilli MC: **HLA-DQ and risk gradient for celiac disease**. *Human immunology* 2009, **70**(1):55-59.

31. Karinen H, Karkkainen P, Pihlajamaki J, Janatuinen E, Heikkinen M, Julkunen R, Kosma VM, Naukkarinen A, Laakso M: **Gene dose effect of the DQB1*0201 allele contributes to severity of coeliac disease**. *Scandinavian journal of gastroenterology* 2006, **41**(2):191-199.

32. Ploski R, Ek J, Thorsby E, Sollid LM: **On the HLA-DQ(alpha 1*0501, beta 1*0201)-associated susceptibility in celiac disease: a possible gene dosage effect of DQB1*0201**. *Tissue antigens* 1993, **41**(4):173-177.

33. Congia M, Cucca F, Frau F, Lampis R, Melis L, Clemente MG, Cao A, De Virgiliis S: **A gene dosage effect of the DQA1*0501/DQB1*0201 allelic combination influences the clinical heterogeneity of celiac disease**. *Human immunology* 1994, **40**(2):138-142.

34. Liu E, Lee HS, Aronsson CA, Hagopian WA, Koletzko S, Rewers MJ, Eisenbarth GS, Bingley PJ, Bonifacio E, Simell V *et al*: **Risk of pediatric celiac disease according to HLA haplotype and country**. *The New England journal of medicine* 2014, **371**(1):42-49.

35. Sandstrom O, Rosen A, Lagerqvist C, Carlsson A, Hernell O, Hogberg L, Ivarsson A: **Transglutaminase IgA antibodies in a celiac disease mass screening and the role of HLA-DQ genotyping and endomysial antibodies in sequential testing**. *Journal of pediatric gastroenterology and nutrition* 2013, **57**(4):472-476.

36. Dubois PC, Trynka G, Franke L, Hunt KA, Romanos J, Curtotti A, Zhernakova A, Heap GA, Adany R, Aromaa A *et al*: **Multiple common variants for celiac disease influencing immune gene expression**. *Nature genetics* 2010, **42**(4):295-302.

37. Schuppan D, Junker Y, Barisani D: **Celiac disease: from pathogenesis to novel therapies**. *Gastroenterology* 2009, **137**(6):1912-1933.

38. Nistico L, Fagnani C, Coto I, Percopo S, Cotichini R, Limongelli MG, Paparo F, D'Alfonso S, Giordano M, Sferlazzas C *et al*: **Concordance, disease progression, and heritability of coeliac disease in Italian twins**. *Gut* 2006, **55**(6):803-808.

39. Greco L, Romino R, Coto I, Di Cosmo N, Percopo S, Maglio M, Paparo F, Gasperi V, Limongelli MG, Cotichini R *et al*: **The first large population based twin study of coeliac disease**. *Gut* 2002, **50**(5):624-628.

40. Jacobson DL, Gange SJ, Rose NR, Graham NM: **Epidemiology and estimated population burden of selected autoimmune diseases in the United States**. *Clinical immunology and immunopathology* 1997, **84**(3):223-243.

41. Wraith DC, Goldman M, Lambert PH: **Vaccination and autoimmune disease: what is the evidence?** *Lancet* 2003, **362**(9396):1659-1666.

42. Jabri B, Sollid LM: **Tissue-mediated control of immunopathology in coeliac disease**. *Nature reviews Immunology* 2009, **9**(12):858-870.

43. Janeway CA, Jr.: **The immune system evolved to discriminate infectious nonself from noninfectious self**. *Immunology today* 1992, **13**(1):11-16.

44. Matzinger P: **Friendly and dangerous signals: is the tissue in control?** *Nature immunology* 2007, **8**(1):11-13.

45. Bernardo D: **Human intestinal dendritic cells as controllers of mucosal immunity**. *Revista espanola de enfermedades digestivas : organo oficial de la Sociedad Espanola de Patologia Digestiva* 2013, **105**(5):279-290.

46. Smith CM, Wilson NS, Waithman J, Villadangos JA, Carbone FR, Heath WR, Belz GT: **Cognate CD4(+) T cell licensing of dendritic cells in CD8(+) T cell immunity**. *Nature immunology* 2004, **5**(11):1143-1148.

47. Shewry PR, Halford NG, Belton PS, Tatham AS: **The structure and properties of gluten: an elastic protein from wheat grain**. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 2002, **357**(1418):133-142.

48. Shewry PR: **Wheat**. *Journal of experimental botany* 2009, **60**(6):1537-1553.

49. Shewry PR, Halford NG: **Cereal seed storage proteins: structures, properties and role in grain utilization**. *Journal of experimental botany* 2002, **53**(370):947-958.

50. Tjon JM, van Bergen J, Koning F: **Celiac disease: how complicated can it get?** *Immunogenetics* 2010, **62**(10):641-651.

51. Kagnoff MF: **Celiac disease: pathogenesis of a model immunogenetic disease**. *The Journal of clinical investigation* 2007, **117**(1):41-49.

52. Arentz-Hansen H, Fleckenstein B, Molberg O, Scott H, Koning F, Jung G, Roepstorff P, Lundin KE, Sollid LM: **The molecular basis for oat intolerance in patients with celiac disease**. *PLoS medicine* 2004, **1**(1):e1.

53. Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C: **Structural basis for gluten intolerance in celiac sprue**. *Science* 2002, **297**(5590):2275-2279.

54. Shan L, Qiao SW, Arentz-Hansen H, Molberg O, Gray GM, Sollid LM, Khosla C: **Identification and analysis of multivalent proteolytically resistant peptides from gluten: implications for celiac sprue**. *Journal of proteome research* 2005, **4**(5):1732-1741.

55. Jabri B, Ebert E: **Human CD8+ intraepithelial lymphocytes: a unique model to study the regulation of effector cytotoxic T lymphocytes in tissue**. *Immunological reviews* 2007, **215**:202-214.

56. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T *et al*: **Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease**. *Immunity* 2004, **21**(3):357-366.

57. Ciccocioppo R, Di Sabatino A, Bauer M, Della Riccia DN, Bizzini F, Biagi F, Cifone MG, Corazza GR, Schuppan D: **Matrix metalloproteinase pattern in celiac duodenal mucosa**. *Laboratory investigation; a journal of technical methods and pathology* 2005, **85**(3):397-407.

58. Monteleone G, Pender SL, Alstead E, Hauer AC, Lionetti P, McKenzie C, MacDonald TT: **Role of interferon alpha in promoting T helper cell type 1 responses in the small intestine in coeliac disease**. *Gut* 2001, **48**(3):425-429.

59. Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, Jahnsen J, Scott H, Brandtzaeg P: **Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease**. *Gastroenterology* 1998, **115**(3):551-563.

60. Lorand L, Graham RM: **Transglutaminases: crosslinking enzymes with pleiotropic functions**. *Nature reviews Molecular cell biology* 2003, **4**(2):140-156.

61. Sollid LM: **Coeliac disease: dissecting a complex inflammatory disorder**. *Nature reviews Immunology* 2002, **2**(9):647-655.

62. Stene LC, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L, Taki I, Norris JM, Erlich HA, Eisenbarth GS *et al*: **Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study**. *The American journal of gastroenterology* 2006, **101**(10):2333-2340.

63. Myleus A, Hernell O, Gothefors L, Hammarstrom ML, Persson LA, Stenlund H, Ivarsson A: **Early infections are associated with increased risk for celiac disease: an incident case-referent study**. *BMC pediatrics* 2012, **12**:194.

64. Welander A, Tjernberg AR, Montgomery SM, Ludvigsson J, Ludvigsson JF: **Infectious disease and risk of later celiac disease in childhood**. *Pediatrics* 2010, **125**(3):e530-536.

65. Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, Gajer P, White JR, Koenig SS, Sakamoto J, Boothe D *et al*: **Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants**. *PloS one* 2012, **7**(3):e33387.

66. Sjoberg V, Sandstrom O, Hedberg M, Hammarstrom S, Hernell O, Hammarstrom ML: **Intestinal T-cell responses in celiac disease - impact of celiac disease associated bacteria**. *PloS one* 2013, **8**(1):e53414.

67. Cheng J, Kalliomaki M, Heilig HG, Palva A, Lahteenoja H, de Vos WM, Salojarvi J, Satokari R: **Duodenal microbiota composition and mucosal homeostasis in pediatric celiac disease**. *BMC gastroenterology* 2013, **13**:113.

68. Schnitzer ME, van der Laan MJ, Moodie EE, Platt RW: **Effect of Breastfeeding on Gastrointestinal Infection in Infants: A Targeted Maximum Likelihood Approach for Clustered Longitudinal Data**. *The annals of applied statistics* 2014, **8**(2):703-725.

69. Akobeng AK, Ramanan AV, Buchan I, Heller RF: **Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies**. *Archives of disease in childhood* 2006, **91**(1):39-43.

70. Norris JM, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE, Emery LM, Sokol RJ, Erlich HA, Eisenbarth GS *et al*: **Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease**. *JAMA : the journal of the American Medical Association* 2005, **293**(19):2343-2351.

71. Poole JA, Barriga K, Leung DY, Hoffman M, Eisenbarth GS, Rewers M, Norris JM: **Timing of initial exposure to cereal grains and the risk of wheat allergy**. *Pediatrics* 2006, **117**(6):2175-2182.

72. World Health Organization: **The optimal duartion of exclusive breastfeeding: report of an expert consultation.** *(Accessed July 19, 2012 at www.who.int)* 2001.

73. Prescott SL, Smith P, Tang M, Palmer DJ, Sinn J, Huntley SJ, Cormack B, Heine RG, Gibson RA, Makrides M: **The importance of early complementary feeding in the development of oral tolerance: concerns and controversies**. *Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology* 2008, **19**(5):375-380.

74. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y: **The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction**. *J Immunol* 1997, **159**(4):1739-1745.

75. Rampertab SD, Pooran N, Brar P, Singh P, Green PH: **Trends in the presentation of celiac disease**. *The American journal of medicine* 2006, **119**(4):355 e359-314.

76. Telega G, Bennet TR, Werlin S: **Emerging new clinical patterns in the presentation of celiac disease**. *Archives of pediatrics & adolescent medicine* 2008, **162**(2):164-168.

77. Olen O, Montgomery SM, Marcus C, Ekbom A, Ludvigsson JF: **Coeliac disease and body mass index: a study of two Swedish general population-based registers**. *Scandinavian journal of gastroenterology* 2009, **44**(10):1198-1206.

78. Dickey W, Kearney N: **Overweight in celiac disease: prevalence, clinical characteristics, and effect of a gluten-free diet**. *The American journal of gastroenterology* 2006, **101**(10):2356-2359.

79. Cheng J, Brar PS, Lee AR, Green PH: **Body mass index in celiac disease: beneficial effect of a gluten-free diet**. *Journal of clinical gastroenterology* 2010, **44**(4):267-271.

80. Ukkola A, Maki M, Kurppa K, Collin P, Huhtala H, Kekkonen L, Kaukinen K: **Changes in body mass index on a gluten-free diet in coeliac disease: a nationwide study**. *European journal of internal medicine* 2012, **23**(4):384-388.

81. Kelly CP, Bai JC, Liu E, Leffler DA: **Advances in Diagnosis and Management of Celiac Disease**. *Gastroenterology* 2015.

82. Ludvigsson JF, Ansved P, Falth-Magnusson K, Hammersjo JA, Johansson C, Edvardsson S, Ljungkrantz M, Stenhammar L, Ludvigsson J: **Symptoms and signs have changed in Swedish children with coeliac disease**. *Journal of pediatric gastroenterology and nutrition* 2004, **38**(2):181-186.

83. Branski D, Troncone R: **Celiac disease: a reappraisal**. *The Journal of pediatrics* 1998, **133**(2):181-187.

84. Briani C, Zara G, Alaedini A, Grassivaro F, Ruggero S, Toffanin E, Albergoni MP, Luca M, Giometto B, Ermani M *et al*: **Neurological complications of celiac disease and autoimmune mechanisms: a prospective study**. *Journal of neuroimmunology* 2008, **195**(1-2):171-175.

85. Ludvigsson JF, Sellgren C, Runeson B, Langstrom N, Lichtenstein P: **Increased suicide risk in coeliac disease--a Swedish nationwide cohort study**. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2011, **43**(8):616-622.

86. Krzywicka B, Herman K, Kowalczyk-Zajac M, Pytrus T: **Celiac disease and its impact on the oral health status - review of the literature**. *Advances in clinical and experimental medicine : official organ Wroclaw Medical University* 2014, **23**(5):675-681.

87. Fouda MA, Khan AA, Sultan MS, Rios LP, McAssey K, Armstrong D: **Evaluation and management of skeletal health in celiac disease: position statement**. *Canadian journal of gastroenterology = Journal canadien de gastroenterologie* 2012, **26**(11):819-829.

88. Murray JA, Van Dyke C, Plevak MF, Dierkhising RA, Zinsmeister AR, Melton LJ, 3rd: **Trends in the identification and clinical features of celiac disease in a North American community, 1950-2001**. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2003, **1**(1):19-27.

89. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C *et al*: **European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease**. *Journal of pediatric gastroenterology and nutrition* 2012, **54**(1):136-160.

90. Kabbani TA, Goldberg A, Kelly CP, Pallav K, Tariq S, Peer A, Hansen J, Dennis M, Leffler DA: **Body mass index and the risk of obesity in coeliac disease treated with the gluten-free diet**. *Alimentary pharmacology & therapeutics* 2012, **35**(6):723-729.

91. Brambilla P, Picca M, Dilillo D, Meneghin F, Cravidi C, Tischer MC, Vivaldo T, Bedogni G, Zuccotti GV: **Changes of body mass index in celiac children on a gluten-free diet**. *Nutrition, metabolism, and cardiovascular diseases : NMCD* 2013, **23**(3):177-182.

92. Haapalahti M, Kulmala P, Karttunen TJ, Paajanen L, Laurila K, Maki M, Mykkanen H, Kokkonen J: **Nutritional status in adolescents and young adults with screen-detected celiac disease**. *Journal of pediatric gastroenterology and nutrition* 2005, **40**(5):566-570.

93. Hoffenberg EJ, Emery LM, Barriga KJ, Bao F, Taylor J, Eisenbarth GS, Haas JE, Sokol RJ, Taki I, Norris JM *et al*: **Clinical features of children with screening-identified evidence of celiac disease**. *Pediatrics* 2004, **113**(5):1254-1259.

94. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID *et al*: **Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study**. *Archives of internal medicine* 2003, **163**(3):286-292.

95. Cataldo F, Marino V: **Increased prevalence of autoimmune diseases in first-degree relatives of patients with celiac disease**. *Journal of pediatric gastroenterology and nutrition* 2003, **36**(4):470-473.

96. Viljamaa M, Kaukinen K, Huhtala H, Kyronpalo S, Rasmussen M, Collin P: **Coeliac disease, autoimmune diseases and gluten exposure**. *Scandinavian journal of gastroenterology* 2005, **40**(4):437-443.

97. Neuhausen SL, Steele L, Ryan S, Mousavi M, Pinto M, Osann KE, Flodman P, Zone JJ: **Co-occurrence of celiac disease and other autoimmune diseases in celiacs and their first-degree relatives**. *Journal of autoimmunity* 2008, **31**(2):160-165.

98. Emilsson L, Wijmenga C, Murray JA, Ludvigsson JF: **Autoimmune Disease in First-degree Relatives and Spouses of Individuals with Celiac Disease**. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2015.

99. Green PH: **The many faces of celiac disease: clinical presentation of celiac disease in the adult population**. *Gastroenterology* 2005, **128**(4 Suppl 1):S74-78.

100. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Pivor M *et al*: **Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition**. *Journal of pediatric gastroenterology and nutrition* 2005, **40**(1):1-19.

101. Jakes AD, Bradley S, Donlevy L: **Dermatitis herpetiformis**. *BMJ* 2014, **348**:g2557.

102. Counsell CE, Taha A, Ruddell WS: **Coeliac disease and autoimmune thyroid disease**. *Gut* 1994, **35**(6):844-846.

103. Sategna-Guidetti C, Bruno M, Mazza E, Carlino A, Predebon S, Tagliabue M, Brossa C: **Autoimmune thyroid diseases and coeliac disease**. *European journal of gastroenterology & hepatology* 1998, **10**(11):927-931.

104. Collin P, Kaukinen K, Valimaki M, Salmi J: **Endocrinological disorders and celiac disease**. *Endocrine reviews* 2002, **23**(4):464-483.

105. Meloni A, Mandas C, Jores RD, Congia M: **Prevalence of autoimmune thyroiditis in children with celiac disease and effect of gluten withdrawal**. *The Journal of pediatrics* 2009, **155**(1):51-55, 55 e51.

106. Ch'ng CL, Jones MK, Kingham JG: **Celiac disease and autoimmune thyroid disease**. *Clinical medicine & research* 2007, **5**(3):184-192.

107. Volta U, Ravaglia G, Granito A, Forti P, Maioli F, Petrolini N, Zoli M, Bianchi FB: **Coeliac disease in patients with autoimmune thyroiditis**. *Digestion* 2001, **64**(1):61-65.

108. Naiyer AJ, Shah J, Hernandez L, Kim SY, Ciaccio EJ, Cheng J, Manavalan S, Bhagat G, Green PH: **Tissue transglutaminase antibodies in individuals with celiac disease bind to thyroid follicles and extracellular matrix and may contribute to thyroid dysfunction**. *Thyroid : official journal of the American Thyroid Association* 2008, **18**(11):1171-1178.

109. Scofield RH: **Autoantibodies as predictors of disease**. *Lancet* 2004, **363**(9420):1544-1546.

110. Roberts CG, Ladenson PW: **Hypothyroidism**. *Lancet* 2004, **363**(9411):793-803.

111. Prummel MF, Wiersinga WM: **Thyroid peroxidase autoantibodies in euthyroid subjects**. *Best practice & research Clinical endocrinology & metabolism* 2005, **19**(1):1-15.

112. Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, Grimley Evans J, Hasan DM, Rodgers H, Tunbridge F *et al*: **The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey**. *Clinical endocrinology* 1995, **43**(1):55-68.

113. Baker JR, Jr.: **Autoimmune endocrine disease**. *JAMA : the journal of the American Medical Association* 1997, **278**(22):1931-1937.

114. Ventura A, Magazzu G, Greco L: **Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. SIGEP Study Group for Autoimmune Disorders in Celiac Disease**. *Gastroenterology* 1999, **117**(2):297-303.

115. Ventura A, Neri E, Ughi C, Leopaldi A, Citta A, Not T: **Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease**. *The Journal of pediatrics* 2000, **137**(2):263-265.

116. Oderda G, Rapa A, Zavallone A, Strigini L, Bona G: **Thyroid autoimmunity in childhood celiac disease**. *Journal of pediatric gastroenterology and nutrition* 2002, **35**(5):704-705.

117. Sategna Guidetti C, Solerio E, Scaglione N, Aimo G, Mengozzi G: **Duration of gluten exposure in adult coeliac disease does not correlate with the risk for autoimmune disorders**. *Gut* 2001, **49**(4):502-505.

118. Sategna-Guidetti C, Volta U, Ciacci C, Usai P, Carlino A, De Franceschi L, Camera A, Pelli A, Brossa C: **Prevalence of thyroid disorders in untreated adult celiac disease patients and effect of gluten withdrawal: an Italian multicenter study**. *The American journal of gastroenterology* 2001, **96**(3):751-757.

119. Biagi F, Pezzimenti D, Campanella J, Corazza GR: **Gluten exposure and risk of autoimmune disorders**. *Gut* 2002, **51**(1):140-141.

120. Radetti G, Maselli M, Buzi F, Corrias A, Mussa A, Cambiaso P, Salerno M, Cappa M, Baiocchi M, Gastaldi R *et al*: **The natural history of the normal/mild elevated TSH serum levels in children and adolescents with Hashimoto's thyroiditis and isolated hyperthyrotropinaemia: a 3-year follow-up**. *Clinical endocrinology* 2012, **76**(3):394-398.

121. Diamanti A, Ferretti F, Guglielmi R, Panetta F, Colistro F, Cappa M, Daniele A, Sole Basso M, Noto C, Crisogianni M *et al*: **Thyroid autoimmunity in children with coeliac disease: a prospective survey**. *Archives of disease in childhood* 2011, **96**(11):1038-1041.

122. Leffler DA, Schuppan D: **Update on serologic testing in celiac disease**. *The American journal of gastroenterology* 2010, **105**(12):2520-2524.

123. Tesei N, Sugai E, Vazquez H, Smecuol E, Niveloni S, Mazure R, Moreno ML, Gomez JC, Maurino E, Bai JC: **Antibodies to human recombinant tissue transglutaminase may detect coeliac disease patients undiagnosed by endomysial antibodies**. *Alimentary pharmacology & therapeutics* 2003, **17**(11):1415-1423.

124. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA: **ACG clinical guidelines: diagnosis and management of celiac disease**. *The American journal of gastroenterology* 2013, **108**(5):656-676; quiz 677.

125. Basso D, Guariso G, Fogar P, Meneghel A, Zambon CF, Navaglia F, Greco E, Schiavon S, Rugge M, Plebani M: **Antibodies against synthetic deamidated gliadin peptides for celiac disease diagnosis and follow-up in children**. *Clinical chemistry* 2009, **55**(1):150-157.

126. Amarri S, Alvisi P, De Giorgio R, Gelli MC, Cicola R, Tovoli F, Sassatelli R, Caio G, Volta U: **Antibodies to deamidated gliadin peptides: an accurate predictor of coeliac disease in infancy**. *Journal of clinical immunology* 2013, **33**(5):1027-1030.

127. Branski D, Faber J, Freier S, Gottschalk-Sabag S, Shiner M: **Histologic evaluation of endoscopic versus suction biopsies of small intestinal mucosae in children with and without celiac disease**. *Journal of pediatric gastroenterology and nutrition* 1998, **27**(1):6-11.

128. Levinson-Castiel R, Hartman C, Morgenstern S, Avitzur Y, Hirsch A, Rosenbach Y, Shapiro R, Zevit N, Fleischaker H, Niv Y *et al*: **The role of duodenal bulb biopsy in the diagnosis of celiac disease in children**. *Journal of clinical gastroenterology* 2011, **45**(1):26-29.

129. Dickey W: **Endoscopic markers for celiac disease**. *Nature clinical practice Gastroenterology & hepatology* 2006, **3**(10):546-551.

130. Kasirer Y, Turner D, Lerman L, Schechter A, Waxman J, Dayan B, Bergwerk A, Rachman Y, Freier Z, Silbermintz A: **Scalloping is a reliable endoscopic marker for celiac disease**. *Digestive endoscopy : official journal of the Japan Gastroenterological Endoscopy Society* 2014, **26**(2):232-235.

131. Oberhuber G, Granditsch G, Vogelsang H: **The histopathology of coeliac disease: time for a standardized report scheme for pathologists**. *European journal of gastroenterology & hepatology* 1999, **11**(10):1185-1194.

132. Sollid LM, Khosla C: **Novel therapies for coeliac disease**. *Journal of internal medicine* 2011, **269**(6):604-613.

133. Olsson C, Lyon P, Hornell A, Ivarsson A, Sydner YM: **Food that makes you different: the stigma experienced by adolescents with celiac disease**. *Qualitative health research* 2009, **19**(7):976-984.

134. Lee SK, Lo W, Memeo L, Rotterdam H, Green PH: **Duodenal histology in patients with celiac disease after treatment with a gluten-free diet**. *Gastrointestinal endoscopy* 2003, **57**(2):187-191.

135. Norstrom F, Sandstrom O, Lindholm L, Ivarsson A: **A gluten-free diet effectively reduces symptoms and health care consumption in a Swedish celiac disease population**. *BMC gastroenterology* 2012, **12**(1):125.

136. Kautto E, Ivarsson A, Norstrom F, Hogberg L, Carlsson A, Hornell A: **Nutrient intake in adolescent girls and boys diagnosed with coeliac disease at an early age is mostly comparable to their non-coeliac contemporaries**. *Journal of human nutrition and dietetics : the official journal of the British Dietetic Association* 2014, **27**(1):41-53.

137. Myleus A, Petersen S, Carlsson A, Hammarroth S, Hogberg L, Ivarsson A: **Health-related quality of life is not impaired in children with undetected as well as diagnosed celiac disease: a large population based cross-sectional study**. *BMC public health* 2014, **14**:425.

138. Panzer RM, Dennis M, Kelly CP, Weir D, Leichtner A, Leffler DA: **Navigating the gluten-free diet in college**. *Journal of pediatric gastroenterology and nutrition* 2012, **55**(6):740-744.

139. Ukkola A, Maki M, Kurppa K, Collin P, Huhtala H, Kekkonen L, Kaukinen K: **Patients' experiences and perceptions of living with coeliac disease - implications for optimizing care**. *Journal of gastrointestinal and liver diseases : JGLD* 2012, **21**(1):17-22.

140. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH, Hadjivassiliou M, Kaukinen K, Kelly CP, Leonard JN *et al*: **The Oslo definitions for coeliac disease and related terms**. *Gut* 2013, **62**(1):43-52.

141. Leffler DA, Dennis M, Hyett B, Kelly E, Schuppan D, Kelly CP: **Etiologies and predictors of diagnosis in nonresponsive celiac disease**. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2007, **5**(4):445-450.

142. O'Mahony S, Howdle PD, Losowsky MS: **Review article: management of patients with non-responsive coeliac disease**. *Alimentary pharmacology & therapeutics* 1996, **10**(5):671-680.

143. Lohi S, Maki M, Rissanen H, Knekt P, Reunanen A, Kaukinen K: **Prognosis of unrecognized coeliac disease as regards mortality: a population-based cohort study**. *Annals of medicine* 2009, **41**(7):508-515.

144. Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD *et al*: **Increased prevalence and mortality in undiagnosed celiac disease**. *Gastroenterology* 2009, **137**(1):88-93.

145. Viljamaa M, Kaukinen K, Pukkala E, Hervonen K, Reunala T, Collin P: **Malignancies and mortality in patients with coeliac disease and dermatitis herpetiformis: 30-year population-based study**. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2006, **38**(6):374-380.

146. West J, Logan RF, Smith CJ, Hubbard RB, Card TR: **Malignancy and mortality in people with coeliac disease: population based cohort study**. *BMJ* 2004, **329**(7468):716-719.

147. Ludvigsson JF, Montgomery SM, Ekbom A, Brandt L, Granath F: **Small-intestinal histopathology and mortality risk in celiac disease**. *JAMA : the journal of the American Medical Association* 2009, **302**(11):1171-1178.

148. Green PH: **Mortality in celiac disease, intestinal inflammation, and gluten sensitivity**. *JAMA : the journal of the American Medical Association* 2009, **302**(11):1225-1226.

149. Saadah OI, Zacharin M, O'Callaghan A, Oliver MR, Catto-Smith AG: **Effect of gluten-free diet and adherence on growth and diabetic control in diabetics with coeliac disease**. *Archives of disease in childhood* 2004, **89**(9):871-876.

150. Leeds JS, Hopper AD, Hadjivassiliou M, Tesfaye S, Sanders DS: **High prevalence of microvascular complications in adults with type 1 diabetes and newly diagnosed celiac disease**. *Diabetes care* 2011, **34**(10):2158-2163.

151. Hogg-Kollars S, Al Dulaimi D, Tait K, Rostami K: **Type 1 diabetes mellitus and gluten induced disorders**. *Gastroenterology and hepatology from bed to bench* 2014, **7**(4):189-197.

152. Trier JS: **Celiac sprue**. *The New England journal of medicine* 1991, **325**(24):1709-1719.

153. Rubio-Tapia A, Murray JA: **Classification and management of refractory coeliac disease**. *Gut* 2010, **59**(4):547-557.

154. Olen O, Askling J, Ludvigsson JF, Hildebrand H, Ekbom A, Smedby KE: **Coeliac disease characteristics, compliance to a gluten free diet and risk of lymphoma by subtype**. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2011, **43**(11):862-868.

155. Vader W, Stepniak D, Kooy Y, Mearin L, Thompson A, van Rood JJ, Spaenij L, Koning F: **The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T cell responses**. *Proceedings of the National Academy of Sciences of the United States of America* 2003, **100**(21):12390-12395.

156. Aggarwal S, Lebwohl B, Green PH: **Screening for celiac disease in average-risk and high-risk populations**. *Therapeutic advances in gastroenterology* 2012, **5**(1):37-47.

157. Lebwohl B, Rubio-Tapia A, Assiri A, Newland C, Guandalini S: **Diagnosis of celiac disease**. *Gastrointestinal endoscopy clinics of North America* 2012, **22**(4):661-677.

158. Fasano A, Catassi C: **Clinical practice. Celiac disease**. *The New England journal of medicine* 2012, **367**(25):2419-2426.

159. Hogberg L, Falth-Magnusson K, Grodzinsky E, Stenhammar L: **Familial prevalence of coeliac disease: a twenty-year follow-up study**. *Scandinavian journal of gastroenterology* 2003, **38**(1):61-65.

160. Catassi C, Kryszak D, Bhatti B, Sturgeon C, Helzlsouer K, Clipp SL, Gelfond D, Puppa E, Sferruzza A, Fasano A: **Natural history of celiac disease autoimmunity in a USA cohort followed since 1974**. *Annals of medicine* 2010, **42**(7):530-538.

161. Fasano A, Catassi C: **Coeliac disease in children**. *Best practice & research Clinical gastroenterology* 2005, **19**(3):467-478.

162. Ivarsson A, Persson LA, Nystrom L, Ascher H, Cavell B, Danielsson L, Dannaeus A, Lindberg T, Lindquist B, Stenhammar L *et al*: **Epidemic of coeliac disease in Swedish children**. *Acta Paediatr* 2000, **89**(2):165-171.

163. Ivarsson A, Hogberg L, Stenhammar L: **The Swedish Childhood Coeliac Disease Working Group after 20 years: history and future**. *Acta Paediatr* 2010, **99**(9):1429-1431.

164. Olsson C, Hernell O, Hornell A, Lonnberg G, Ivarsson A: **Difference in celiac disease risk between Swedish birth cohorts suggests an opportunity for primary prevention**. *Pediatrics* 2008, **122**(3):528-534.

165. Ivarsson A, Hernell O, Stenlund H, Persson LA: **Breast-feeding protects against celiac disease**. *The American journal of clinical nutrition* 2002, **75**(5):914-921.

166. **ESPGAN committee on nutrition. Guidelines on infant nutrition. III. Recommendations for infant feeding**. *Acta paediatrica Scandinavica Supplement* 1982, **302**:1-27.

167. Silano M, Agostoni C, Guandalini S: **Effect of the timing of gluten introduction on the development of celiac disease**. *World J Gastroenterol* 2010, **16**(16):1939-1942.

168. Hogen Esch CE, Rosen A, Auricchio R, Romanos J, Chmielewska A, Putter H, Ivarsson A, Szajewska H, Koning F, Wijmenga C *et al*: **The PreventCD Study design: towards new strategies for the prevention of coeliac disease**. *European journal of gastroenterology & hepatology* 2010, **22**(12):1424-1430.

169. Webb C, Halvarsson B, Norstrom F, Myleus A, Carlsson A, Danielsson L, Hogberg L, Ivarsson A, Karlsson E, Stenhammar L *et al*: **Accuracy in celiac disease diagnostics by controlling the small-bowel biopsy process**. *Journal of pediatric gastroenterology and nutrition* 2011, **52**(5):549-553.

170. Dickson BC, Streutker CJ, Chetty R: **Coeliac disease: an update for pathologists**. *Journal of clinical pathology* 2006, **59**(10):1008-1016.

171. Ivarsson A MA, Wall S: **Towards preventing celiac disease - an epidemiological approach**. *Fasano A, Troncone R, Branski D, eds Pediatric and Adolescent Medicine New Frontiers in Celiac Disease Karger Publishers, Basel, Switzerland* 2008:198-209.

172. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH: **Establishing a standard definition for child overweight and obesity worldwide: international survey**. *BMJ* 2000, **320**(7244):1240-1243.

173. Cole TJ, Flegal KM, Nicholls D, Jackson AA: **Body mass index cut offs to define thinness in children and adolescents: international survey**. *BMJ* 2007, **335**(7612):194.

174. Surks MI, Chopra IJ, Mariash CN, Nicoloff JT, Solomon DH: **American Thyroid Association guidelines for use of laboratory tests in thyroid disorders**. *JAMA : the journal of the American Medical Association* 1990, **263**(11):1529-1532.

175. Abramson JH: **WINPEPI updated: computer programs for epidemiologists, and their teaching potential**. *Epidemiologic perspectives & innovations : EP+I* 2011, **8**(1):1.

177. Mamdani M, Sykora K, Li P, Normand SL, Streiner DL, Austin PC, Rochon PA, Anderson GM: **Reader's guide to critical appraisal of cohort studies: 2. Assessing potential for confounding**. *BMJ* 2005, **330**(7497):960-962.

178. Rochon PA, Gurwitz JH, Sykora K, Mamdani M, Streiner DL, Garfinkel S, Normand SL, Anderson GM: **Reader's guide to critical appraisal of cohort studies: 1. Role and design**. *BMJ* 2005, **330**(7496):895-897.

179. Bonita R BR, Kjellström T: **Basic Epidemiology. 2nd edition**. *India: World Health Organisation (WHO Press); 2006* 2006.

180. Carlson MD, Morrison RS: **Study design, precision, and validity in observational studies**. *Journal of palliative medicine* 2009, **12**(1):77-82.

181. Ivarsson A: **The Swedish epidemic of coeliac disease explored using an epidemiological approach--some lessons to be learnt**. *Best practice & research Clinical gastroenterology* 2005, **19**(3):425-440.

182. Rothman KJ GS: **Modern Epidemiology, Second Edition.** . *Philadelphia, PA: Lippincott-Raven, 1998* 1998.

183. Kurppa K, Collin P, Maki M, Kaukinen K: **Celiac disease and health-related quality of life**. *Expert review of gastroenterology & hepatology* 2011, **5**(1):83-90.

184. Norstrom F, Lindholm L, Sandstrom O, Nordyke K, Ivarsson A: **Delay to celiac disease diagnosis and its implications for health-related quality of life**. *BMC gastroenterology* 2011, **11**:118.

185. Nordyke K, Norstrom F, Lindholm L, Carlsson A, Danielsson L, Emmelin M, Hogberg L, Karlsson E, Ivarsson A: **Health-related quality-of-life in children with coeliac disease, measured prior to receiving their diagnosis through screening**. *Journal of medical screening* 2011.

186. D'Amico MA, Holmes J, Stavropoulos SN, Frederick M, Levy J, DeFelice AR, Kazlow PG, Green PH: **Presentation of pediatric celiac disease in the United States: prominent effect of breastfeeding**. *Clinical pediatrics* 2005, **44**(3):249-258.

187. Radlovic NP, Mladenovic MM, Lekovic ZM, Stojsic ZM, Radlovic VN: **Influence of early feeding practices on celiac disease in infants**. *Croatian medical journal* 2010, **51**(5):417-422.

188. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolacek S, Koletzko B, Michaelsen KF, Moreno L, Puntis J, Rigo J *et al*: **Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition**. *Journal of pediatric gastroenterology and nutrition* 2008, **46**(1):99-110.

189. Agostoni C, Braegger C, Decsi T, Kolacek S, Koletzko B, Michaelsen KF, Mihatsch W, Moreno LA, Puntis J, Shamir R *et al*: **Breast-feeding: A commentary by the ESPGHAN Committee on Nutrition**. *Journal of pediatric gastroenterology and nutrition* 2009, **49**(1):112-125.

190. Greer FR, Sicherer SH, Burks AW: **Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas**. *Pediatrics* 2008, **121**(1):183-191.

191. Carlsson A, Agardh D, Borulf S, Grodzinsky E, Axelsson I, Ivarsson SA: **Prevalence of celiac disease: before and after a national change in feeding recommendations**. *Scandinavian journal of gastroenterology* 2006, **41**(5):553-558.

192. Verhasselt V: **Oral tolerance in neonates: from basics to potential prevention of allergic disease**. *Mucosal immunology* 2010, **3**(4):326-333.

193. Ludvigsson JF, Fasano A: **Timing of introduction of gluten and celiac disease risk**. *Annals of nutrition & metabolism* 2012, **60 Suppl 2**:22-29.

194. Sanchez E, De Palma G, Capilla A, Nova E, Pozo T, Castillejo G, Varea V, Marcos A, Garrote JA, Polanco I *et al*: **Influence of environmental and genetic factors linked to celiac disease risk on infant gut colonization by Bacteroides species**. *Applied and environmental microbiology* 2011, **77**(15):5316-5323.

195. Tjellstrom B, Stenhammar L, Hogberg L, Falth-Magnusson K, Magnusson KE, Midtvedt T, Sundqvist T, Norin E: **Gut microflora associated characteristics in children with celiac disease**. *The American journal of gastroenterology* 2005, **100**(12):2784-2788.

196. Aronsson CA, Lee HS, Liu E, Uusitalo U, Hummel S, Yang J, Hummel M, Rewers M, She JX, Simell O *et al*: **Age at gluten introduction and risk of celiac disease**. *Pediatrics* 2015, **135**(2):239-245.

197. Stordal K, White RA, Eggesbo M: **Early feeding and risk of celiac disease in a prospective birth cohort**. *Pediatrics* 2013, **132**(5):e1202-1209.

198. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S, Barbato M, Barbera C, Barera G, Bellantoni A *et al*: **Introduction of gluten, HLA status, and the risk of celiac disease in children**. *The New England journal of medicine* 2014, **371**(14):1295-1303.

199. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P, Kolacek S, Koletzko S, Korponay-Szabo IR, Mummert E *et al*: **Randomized feeding intervention in infants at high risk for celiac disease**. *The New England journal of medicine* 2014, **371**(14):1304-1315.

200. Catassi C, Rossini M, Ratsch IM, Bearzi I, Santinelli A, Castagnani R, Pisani E, Coppa GV, Giorgi PL: **Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study**. *Gut* 1993, **34**(11):1515-1519.

201. Akobeng AK, Thomas AG: **Systematic review: tolerable amount of gluten for people with coeliac disease**. *Alimentary pharmacology & therapeutics* 2008, **27**(11):1044-1052.

202. Olivares M, Neef A, Castillejo G, Palma GD, Varea V, Capilla A, Palau F, Nova E, Marcos A, Polanco I *et al*: **The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease**. *Gut* 2015, **64**(3):406-417.

203. Welander A, Montgomery S, Ludvigsson J, Ludvigsson JF: **Breast-feeding duration and gluten introduction among mothers with celiac disease**. *Journal of pediatric gastroenterology and nutrition* 2014, **59**(1):89-92.

204. Palma GD, Capilla A, Nova E, Castillejo G, Varea V, Pozo T, Garrote JA, Polanco I, Lopez A, Ribes-Koninckx C *et al*: **Influence of milk-feeding type and genetic risk of developing coeliac disease on intestinal microbiota of infants: the PROFICEL study**. *PloS one* 2012, **7**(2):e30791.

205. Sonti R, Lebwohl B, Lewis SK, Abu Daya H, Klavan H, Aguilar K, Green PH: **Men with celiac disease are shorter than their peers in the general population**. *European journal of gastroenterology & hepatology* 2013, **25**(9):1033-1037.

206. Reilly NR, Aguilar K, Hassid BG, Cheng J, Defelice AR, Kazlow P, Bhagat G, Green PH: **Celiac disease in normal-weight and overweight children: clinical features and growth outcomes following a gluten-free diet**. *Journal of pediatric gastroenterology and nutrition* 2011, **53**(5):528-531.

207. Bingley PJ, Williams AJ, Norcross AJ, Unsworth DJ, Lock RJ, Ness AR, Jones RW: **Undiagnosed coeliac disease at age seven: population based prospective birth cohort study**. *BMJ* 2004, **328**(7435):322-323.

208. Wierdsma NJ, van Bokhorst-de van der Schueren MA, Berkenpas M, Mulder CJ, van Bodegraven AA: **Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients**. *Nutrients* 2013, **5**(10):3975-3992.

209. Nusier MK, Brodtkorb HK, Rein SE, Odeh A, Radaideh AM, Klungland H: **Serological screening for celiac disease in schoolchildren in Jordan. Is height and weight affected when seropositive?** *Italian journal of pediatrics* 2010, **36**:16.

210. Parnanen A, Kaukinen K, Helakorpi S, Uutela A, Lahdeaho ML, Huhtala H, Collin P, Maki M, Kurppa K: **Symptom-detected and screen-detected celiac disease and adult height: a large cohort study**. *European journal of gastroenterology & hepatology* 2012, **24**(9):1066-1070.

211. Damen GM, Boersma B, Wit JM, Heymans HS: **Catch-up growth in 60 children with celiac disease**. *Journal of pediatric gastroenterology and nutrition* 1994, **19**(4):394-400.

212. Luciano A, Bolognani M, Di Falco A, Trabucchi C, Bonetti P, Castellarin A: **[Catch-up growth and final height in celiac disease]**. *La Pediatria medica e chirurgica : Medical and surgical pediatrics* 2002, **24**(1):9-12.

213. Cosnes J, Cosnes C, Cosnes A, Contou JF, Reijasse D, Carbonnel F, Beaugerie L, Gendre JP: **[Undiagnosed celiac disease in childhood]**. *Gastroenterologie clinique et biologique* 2002, **26**(6-7):616-623.

214. Tanner JM, Whitehouse RH, Marshall WA, Carter BS: **Prediction of adult height from height, bone age, and occurrence of menarche, at ages 4 to 16 with allowance for midparent height**. *Archives of disease in childhood* 1975, **50**(1):14-26.

215. Marshall WA, Tanner JM: **Variations in pattern of pubertal changes in girls**. *Archives of disease in childhood* 1969, **44**(235):291-303.

216. Marshall WA, Tanner JM: **Variations in the pattern of pubertal changes in boys**. *Archives of disease in childhood* 1970, **45**(239):13-23.

217. Cosnes J, Cellier C, Viola S, Colombel JF, Michaud L, Sarles J, Hugot JP, Ginies JL, Dabadie A, Mouterde O *et al*: **Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet**. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2008, **6**(7):753-758.

218. van Koppen EJ, Schweizer JJ, Csizmadia CG, Krom Y, Hylkema HB, van Geel AM, Koopman HM, Verloove-Vanhorick SP, Mearin ML: **Long-term health and quality-of-life consequences of mass screening for childhood celiac disease: a 10-year follow-up study**. *Pediatrics* 2009, **123**(4):e582-588.

219. Hopman EG, le Cessie S, von Blomberg BM, Mearin ML: **Nutritional management of the gluten-free diet in young people with celiac disease in The Netherlands**. *Journal of pediatric gastroenterology and nutrition* 2006, **43**(1):102-108.

220. Elfstrom P, Sundstrom J, Ludvigsson JF: **Systematic review with meta-analysis: associations between coeliac disease and type 1 diabetes**. *Alimentary pharmacology & therapeutics* 2014, **40**(10):1123-1132.

221. Fasano A: **Systemic autoimmune disorders in celiac disease**. *Current opinion in gastroenterology* 2006, **22**(6):674-679.

222. Ricano-Ponce I, Wijmenga C: **Mapping of immune-mediated disease genes**. *Annual review of genomics and human genetics* 2013, **14**:325-353.

223. Radetti G, Gottardi E, Bona G, Corrias A, Salardi S, Loche S: **The natural history of euthyroid Hashimoto's thyroiditis in children**. *The Journal of pediatrics* 2006, **149**(6):827-832.

224. Ansaldi N, Palmas T, Corrias A, Barbato M, D'Altiglia MR, Campanozzi A, Baldassarre M, Rea F, Pluvio R, Bonamico M *et al*: **Autoimmune thyroid disease and celiac disease in children**. *Journal of pediatric gastroenterology and nutrition* 2003, **37**(1):63-66.

225. Huber G, Staub JJ, Meier C, Mitrache C, Guglielmetti M, Huber P, Braverman LE: **Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies**. *The Journal of clinical endocrinology and metabolism* 2002, **87**(7):3221-3226.

226. Cassio A, Ricci G, Baronio F, Miniaci A, Bal M, Bigucci B, Conti V, Cicognani A: **Long-term clinical significance of thyroid autoimmunity in children with celiac disease**. *The Journal of pediatrics* 2010, **156**(2):292-295.

227. Wasniewska M, Salerno M, Cassio A, Corrias A, Aversa T, Zirilli G, Capalbo D, Bal M, Mussa A, De Luca F: **Prospective evaluation of the natural course of idiopathic subclinical hypothyroidism in childhood and adolescence**. *European journal of endocrinology / European Federation of Endocrine Societies* 2009, **160**(3):417-421.

228. Zois C, Stavrou I, Svarna E, Seferiadis K, Tsatsoulis A: **Natural course of autoimmune thyroiditis after elimination of iodine deficiency in northwestern Greece**. *Thyroid : official journal of the American Thyroid Association* 2006, **16**(3):289-293.

229. Richard-Miceli C, Criswell LA: **Emerging patterns of genetic overlap across autoimmune disorders**. *Genome medicine* 2012, **4**(1):6.