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Coeliac Disease Beyond Villous Atrophy

ACADEMIC DISSERTATION
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Nec fasces, nec opes, sola artis sceptra perennant

Tyco Brahe

To My Family

ABSTRACT

The diagnosis of coeliac disease is currently based on the demonstration of small-bowel mucosal villous atrophy and crypt hyperplasia. However, the mucosal damage develops gradually and patients may evince clinical symptoms even before the villous atrophy develops. In addition, there are extraintestinal forms of coeliac disease in which the intestinal damage can be milder or even absent. These observations suggest that the current diagnostic criteria are insufficient to cover the wide range of gluten-dependent disorders. The early mucosal changes are too ambivalent for the diagnosis as such, but evidence would indicate that specific coeliac antibodies may also appear prior to villous atrophy. The primary aim in the present study was to establish whether adults and children having positive endomysial antibodies (EmA) suffer from a coeliac-type disorder and would benefit from dietary treatment while still showing normal small-bowel mucosal morphology.

The dissertation comprised altogether four separate clinical studies. Study **I** was a randomized clinical trial involving 70 EmA-positive adults and 34 EmA-negative controls. At baseline, the small-bowel mucosal structure and inflammatory markers, serum coeliac antibodies, clinical symptoms, coeliac disease-associated genotype and laboratory parameters were evaluated in all. A total of 23 EmA-positive subjects were found to have normal mucosal morphology and thus did not fulfil the current diagnostic criteria for coeliac disease. They were randomized either to continue on a normal diet or to start a gluten-free diet. The remaining 47 EmA-positive subjects had villous atrophy and started gluten-free dietary treatment. Finally, after one year on trial all EmA-positive participants were re-evaluated. Study **II** comprised 27 EmA-positive adults with normal mucosal morphology and 46 with villous atrophy. Together with serological and histological evaluations, self-rated gastrointestinal symptoms, health-related quality of life, bone mineral density (BMD) and body mass index (BMI) were measured both at baseline and after one year on a gluten-free diet. Altogether 110 healthy adults served as non-coeliac controls. The cohort in study **III** comprised 17 EmA-positive children having normal villi, 42 children with villous atrophy and 17 EmA-negative controls. After baseline eight EmA-positive children with normal villi continued with a normal diet and five were placed on a gluten-free diet by their parents` decision. Again, all EmA-positive children were re-investigated after one year

on trial. Finally, study **IV** involved three subjects who had coeliac disease with classical gastrointestinal symptoms diagnosed during childhood, but who at some point re-introduced gluten in their diet and after a very long asymptomatic period were remitted to hospital on suspicion of dermatitis herpetiformis.

The results of the prospective studies (**I-III**) were analogous and showed that both EmA-positive adults and children may manifest clinical symptoms and even decreased BMD despite normal small-bowel mucosal structure. In addition, in the EmA-positive subjects who continued on a gluten-containing diet the mucosal changes were exacerbated, coeliac antibody levels increased and clinical symptoms persisted, whereas in those who started treatment the antibodies decreased and clinical symptoms were alleviated. The gluten-dependency of these EmA-positive subjects was further supported by the fact that all had the HLA genotype required for coeliac disease. In Study **II** it was shown that a gluten-free diet may alleviate depression and is not detrimental to the quality of life of EmA-positive subjects with normal villi. The treatment may also improve BMD and would appear not to affect the weight control of these EmA-positive subjects. Finally, skin biopsy in the three cases in Study **IV** confirmed the diagnosis of dermatitis herpetiformis and showed that the coeliac disease phenotype may change after a long asymptomatic period. In addition, although they had no abdominal symptoms and only partial villous atrophy, all three cases had coeliac antibodies in the serum and coeliac-type autoantibody deposition in the intestinal mucosa.

The results of the present series demonstrated that EmA-positive subjects suffer from a gluten-dependent disorder similar to coeliac disease and benefit from treatment despite normal small-bowel mucosal morphology. Furthermore, the clinical and histological presentation of coeliac disease may change over time, showing that the intestinal and extraintestinal forms of the disorder belong to the same category of genetic gluten intolerance. These observations indicate that the current diagnostic criteria for coeliac disease are inadequate and should be revised. In the future more studies are needed to assess whether EmA-positive but asymptomatic patients having normal mucosal morphology should be treated and further, to assess the role of endoscopic studies in the diagnosis of coeliac disease.

TIIVISTELMÄ

Keliakian nykyiset diagnostiset kriteerit edellyttävät ohutsuolen limakalvon suolinukkavaurion osoittamista. Limakalvovaurio kuitenkin kehittyy asteittain, ja potilaat saattavat kärsiä tyypillisistä keliakiaoireista suolinukkarakenteen ollessa vielä normaali. Lisäksi keliakia saattaa esiintyä myös suoliston ulkopuolisina oireyhtyminä, joissa limakalvomuutokset voivat olla lieviä tai puuttua kokonaan. Havainnot viittaavat siihen, että nykyiset diagnostiset kriteerit eivät riitä kattamaan gluteeniriippuvaisten sairauksien laajaa kirjoa. Toisaalta ohutsuolen limakalvon varhaiset muutokset ovat liian epäspesifinen löydös lopullisen keliakiadiagnoosin asettamiseksi. On kuitenkin alustavaa näyttöä, että keliakiaspesifejä vasta-aineita voi esiintyä seerumissa jo ennen suolinukkavaurion kehittymistä. Tämän tutkimuksen tarkoituksena oli arvioida kärsivätkö endomysiinivasta-ainepositiiviset (EmA) henkilöt, joilla on vielä normaali suolinukkarakenne gluteeniriippuvaisesta sairaudesta, sekä hyötyvätkö he varhain aloitetusta keliakian ruokavaliosta.

Väitöskirjatyö koostui kaikkiaan neljästä erillisestä osatyöstä. Osatyö **I** oli satunnaistettu seurantatutkimus, mihin osallistui yhteensä 70 EmA-positiivista aikuista ja 34 EmA-negatiivista verrokkia. Tutkimuksen alussa osallistujille tehtiin laajat histologiset, serologiset ja kliiniset tutkimukset, sekä määritettiin keliakiaan liittyvät perintötekijät. Alkututkimusten perusteella 23 EmA-positiivisella henkilöllä oli vielä normaali suolinukkarakenne. Nämä henkilöt satunnaistettiin joko jatkamaan entisellä ruokavaliollaan tai aloittamaan gluteenivapaa ruokavalio. Lopuilla 47 EmA-positiivisella tutkittavalla todettiin diagnostinen suolinukkavaurio, ja he aloittivat ruokavaliohoidon. Vuoden seurannan jälkeen alkuvaiheen tutkimukset toistettiin kaikille EmA-positiivisille osallistujille. Osatyössä **II** oli kaikkiaan 27 EmA-positiivista aikuista joilla oli normaali suolinukkarakenne, sekä 46 tutkittavaa joilla oli diagnostinen limakalvovaurio. Laajojen histologisten ja serologisten tutkimusten lisäksi osallistujilta määritettiin myös itsearvioidut kliiniset oireet ja terveyteen liittyvä elämänlaatu, sekä mitattiin luuntiheys ja painoindeksi. Yhteensä 110 tervettä aikuista toimi tutkimuksen vertailuryhmänä. Osatyö **III** koostui 17 EmA-postiivisesta lapsesta

joilla oli normaali suolinukka, 42 lapsesta joilla oli suolinukkavaurio, sekä 17 EmA-negatiivisesta verrokista. Alkututkimusten jälkeen viisi EmA-positiivista lasta joilla oli normaali suolinukka sekä lapset joilla oli suolinukkavaurio aloittivat gluteenivapaan ruokavalion. Kahdeksan normaalin suolinukan omaavaa EmA-positiivista lasta jatkoi gluteenipitoisella ruokavaliolla. Vuoden seurannan jälkeen kaikki alkuvaiheen tutkimukset toistettiin. Osatyö **IV** käsitti kolme henkilöä, joilla oli todettu sekä suolinukkavaurio että tyypilliset keliakiaoireet lapsuudessa. Myöhemmin kyseiset henkilöt olivat kuitenkin aloittaneet uudelleen gluteenin käytön, ja pitkän oireettoman ajan jälkeen heidät lähettiin sairaalaan ihokeliakiaepäilyn vuoksi.

Seurantatutkimusten (**I-III**) tulokset osoittivat, että EmA-positiivisilla henkilöillä voi olla keliakiaoireita ja jopa heikentynyt luuntiheys suolinukan ollessa vielä normaali. Lisäksi gluteeninkäyttöä jatkettaessa potilaiden limakalvovaurio paheni, keliakiavasta-ainetasot nousivat ja kliiniset oireet jatkuivat, kun taas ruokavaliohoidon aikana vasta-ainetasot laskivat ja oireet lievittyivät. EmA-positiivisten henkilöiden samankaltaisuutta riippumatta suolinukkavaurion asteesta osoitti myös heiltä jokaiselta todetut keliakiaan sopivat HLA-perintötekijät. Osatyön **II** tulokset osoittivat gluteenivapaan ruokavalion myös lievittävän EmA-positiivisten potilaiden masennusoireita, sekä mahdollisesti parantavan luuntiheyttä jälleen huolimatta normaalista suolinukkarakenteesta. Tulosten perusteella keliakian varhainen hoito ei myöskään huononna EmA-positiivisten aikuisten elämänlaatua tai painonhallintaa. Lopuksi, kaikki kolme potilasta osatyössä **IV** saivat ihokeliakiadiagnoosin, osoittaen että pitkän oireettoman ajanjakson jälkeen keliakian kliininen ja histologinen ilmiasu voivat vaihtua. Lisäksi näillä potilailla todettiin sekä keliakiavasta-aineita seerumissa että keliakiatyyppiset autovasta-ainekertymät ohutsuolen limakalvolla.

Väitökirjatyön tulokset osoittavat EmA-positiivisten henkilöiden kärsivän perinnöllisestä gluteeniherkästä sairaudesta riippumatta ohutsuolen limakalvovaurion asteesta. Lisäksi sekä keliakian kliininen että histologinen taudinkuva voivat muuttua ajan kuluessa, osoittaen klassisen suolioireisen keliakian ja suoliston ulkopuolisen ihokeliakian edustavan saman gluteeniherkän sairauden eri ilmenemismuotoja. Löydösten perusteella keliakian nykyiset diagnostiset kriteerit ovat riittämättömät ja tulisi uudistaa. Tulevaisuudessa tarvitaan vielä lisätutkimuksia päätettäessä tulisiko keliakiavasta-ainepositiivisia täysin oireettomia henkilöitä hoitaa, sekä arvioitaessa ohutsmmuolen tähystystutkimusten asemaa keliakian diagnostiikassa.

CONTENTS

ABBREVIATIONS

AGA	antigliadin antibodies
APC	antigen-presenting cell
ARA	anti-reticulin antibodies
BMI	body mass index
BMD	bone mineral density
CI	confidence interval
CD	Cluster design
CrD	crypt depth
ELISA	enzyme-linked immunosorbent assay
EmA	endomysial antibodies
ESPGAN	European Society of Paediatric Gastroenterology and Nutrition
GSRS	Gastrointestinal Symptom Rating Scale
HLA	human leukocyte antigen
IEL	intraepithelial lymphocyte
Ig	immunoglobulin
IL	interleukin
INF	interferon
ND	no data
PGWB	Psychological General Well-Being
SF-36	Short Form 36 Health Survey
TG	transglutaminase
TG2-ab	transglutaminase 2 antibody
TNF	tumour necrosis factor
U	unit value
Vh	villous height
WHO	World Health Organisation

LIST OF ORIGINAL PUBLICATIOS

This thesis is based on the following original publications, referred to in the text by the Roman numerals **I-IV:**

I Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J, Laurila K, Huhtala H, Paasikivi K, Mäki M, Kaukinen K (2009): Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. Gastroenterology 136:816-23

II Kurppa K, Collin P, Sievänen H, Huhtala H, Mäki M, Kaukinen K (2010): Gastrointestinal symptoms, quality of life and bone mineral density in mild enteropathy coeliac disease: a prospective clinical trial. Scand J Gastroenterol 45:305-14

III Kurppa K, Ashorn M, Iltanen S, Koskinen LL, Saavalainen P, Koskinen O, Mäki M, Kaukinen K. Celiac disease without villous atrophy in children: a prospective study. J Pediatr, DOI: 10.1016/j.peds.2010.02.070

IV Kurppa K, Koskinen O, Collin P, Mäki M, Reunala T, Kaukinen K (2008): Changing phenotype of coeliac disease after long-term gluten exposure. J Pediatr Gastroenterol Nutr 47:500-3

INTRODUCTION

Coeliac disease is a common autoimmune-based disorder caused by ingested gluten in genetically predisposed individuals. The classical symptoms are abdominal complaints, diarrhoea and malabsorption (Gee 1888, Visakorpi et al. 1970), but the clinical symptoms can be heterogeneous and may appear at any age (Mäki et al. 1988a, Murray et al. 2003). The current diagnostic criteria are based on the demonstration of small-bowel mucosal villous atrophy and crypt hyperplasia (Walker-Smith et al. 1990). However, this intestinal damage develops gradually from completely normal mucosa to overt villous atrophy (Marsh 1992) and the deterioration may take years or even decades (Mäki et al. 1990, Lähdeaho et al. 2005). A number of studies have shown that patients may suffer from gluten-dependent symptoms or even complications of coeliac disease before villous atrophy develops (Egan-Mitchell et al. 1981, Collin et al. 1992, Kaukinen et al. 1998, Salmi et al. 2006) and there are also indications that patients may benefit from early treatment with a gluten-free diet even when the mucosal structure is normal (Picarelli et al. 1996a, Kaukinen et al. 2001, Paparo et al. 2005). These results suggest that the current diagnostic criteria for coeliac disease are inadequate and should be revised.

Only a minority of patients with mild small-bowel mucosal changes will eventually develop coeliac disease (Ferguson and Murray 1971, Kuitunen et al. 1982, Kakar 2003, Lähdeaho et al. 2005), and more specific diagnostic methods for early-developing disease are required. During recent decades sensitive and specific endomysial (EmA) and transglutaminase 2 antibodies (TG2-abs) have been increasingly applied in case-finding and as a result subjects with positive antibodies but morphologically normal mucosal villi are frequently detected (Mäki et al. 2003, Fasano et al. 2003). Previously such cases were considered false-positive, but there is evidence that the antibodies may actually predict forthcoming villous atrophy and could be used for an early diagnosis of coeliac disease (Mäki et al. 1991b, Collin et al. 1993, Troncone 1995, Salmi et al. 2006). At present, however, randomized and controlled follow-up trials of the treatment of these antibody-positive patients with

normal small-bowel mucosal villous morphology are lacking and there is no consensus regarding their diagnosis.

The purpose of the present study was to investigate the natural history of coeliac disease in EmA-positive adults and children with normal small-bowel mucosal villous structure. After baseline investigation these EmA-positive subjects either continued with a gluten-containing diet or were placed on an experimental gluten-free diet and were evaluated using a number of clinical, histological and serological markers of coeliac disease. Finally, all results were compared with those obtained in subjects with overt villous atrophy and in non-coeliac controls. In addition, changes in the clinical and histological presentation of coeliac disease over time was investigated in three patients in whom the classical intestinal disorder with small-bowel mucosal villous atrophy had been diagnosed during childhood but who subsequently resumed a gluten-containing diet.

REVIEW OF THE LITERATURE

1. HISTORY OF COELIAC DISEASE

For most of the history of humankind coeliac disease has apparently been a very rare condition (Guandalini 2008). Gluten, the causative agent, is present only in certain cereals which were virtually non-existent in the diet of the early hunter-gatherer societies. Thus, coeliac disease could have evolved only after the development of agriculture in the fertile crescents of South East Asia about 10000 years ago. Even then, the spread of cultivation took thousands of years and, up to the last few centuries, the grains also contained relatively low amounts of gluten. This historical background perhaps explains the conspicuous lack of medical reports of coeliac disease before the last few centuries. Although the Greek physician Aretaeus of Cappadocia provided the first known description about two thousand years ago (Adams 1856), the first medical article appeared as late as 1815 (Baillie 1815) and the earliest systematic report was written by Samuel Gee (1888) just over a hundred years ago.

Although a variety of dietary treatments were attempted during the first half of the 20th century, the actual cause of coeliac disease was unknown and it was often lethal, particularly in children. It was thus a remarkable breakthrough when in the early 1950s it was shown that the trigger of coeliac disease is wheat gluten (Dicke et al. 1953). Soon these after the small-bowel mucosal damage was discovered (Paulley 1954) and several new theories on the pathogenesis began to emerge. In the 1970s the association of coeliac disease with certain genotypes was observed (Stokes et al. 1972) and the wide clinical spectrum and extraintestinal forms of the disorder became evident. During the last few decades the understanding of the pathogenesis has greatly increased and population-based screening studies have revealed that coeliac disease is one of the commonest chronic diseases in the Western world (Mäki et al. 2003, Fasano et al. 2003, Lohi et al. 2007).

2. CLINICAL FEATURES OF COELIAC DISEASE

2.1 Classical gastrointestinal manifestations

What Samuel Gee described as a paediatric disorder with gradual onset of steatorrhea and malnutrition was regarded as the primary manifestation of coeliac disease for almost a hundred years (Gee 1888). Patients often had deficiencies of important trace elements such as iron, calcium, zinc, folate and vitamin B12, and common complications were anaemia, rickets, poor growth, short stature and delayed puberty (Visakorpi et al. 1970, Verkasalo et al. 1978, Mäki et al. 1988a). Although coeliac patients may still evince all these symptoms and complications, it was realized in the 1980s that the clinical presentation is markedly variable and that intestinal symptoms are not always present. Subsequently, older cases and those with milder symptoms were increasingly being recognized (Logan et al. 1983, Mäki et al. 1988a), and nowadays most coeliac patients either suffer from some extraintestinal manifestation or have no symptoms at all (Collin et al. 1997, Murray et al. 2003).

2.2 Extraintestinal manifestations and complications

2.2.1 Dermatitis herpetiformis

Dermatitis herpetiformis is a skin form of coeliac disease (Duhring 1884). It has the same genetic background as the intestinal disease (Katz et al. 1972, Spurkland et al. 1997) and coeliac antibodies are also present in most untreated dermatitis herpetiformis patients (Reunala 2001). The two forms of the disease are associated with the same autoimmune disorders and share many complications such as decreased bone mineral density (BMD) (DiStefano et al. 1999), certain malignancies (Collin et al. 1996a, Askling et al. 2002) and dental enamel defects (Aine 1996). The skin disorder is rare in childhood, the mean age at onset being usually between 30 and 40 years with a slight male predominance, and the characteristic clinical presentation is a blistering rash on elbows and knees, though the rash can appear in all body areas (Reunala 2001). Most dermatitis herpetiformis patients also have either villous atrophy or at least inflammatory changes in the small-bowel mucosa (Marks et al. 1966,

Savilahti et al. 1992), but only a minority suffer from the gastrointestinal symptoms typical of coeliac disease (Reunala 2001).

2.2.2 Other extraintestinal manifestations

Neurological symptoms and complications are common in untreated coeliac disease. Hadjivassiliou and associates (1996) described a series of patients with ataxic symptoms and positive coeliac antibodies, and this so-called gluten ataxia was later taken to refer to subjects with unexplained ataxia and antibodies against gluten-derived gliadin. Although less than half of these cases have small-bowel mucosal villous atrophy, they have the HLA-type characteristic of coeliac disease and may respond to dietary treatment (Hadjivassiliou 2008a). An increased prevalence of epilepsy has also been associated with coeliac disease (Cooke and Smith 1966, Chapman et al. 1978), but the risk would appear to be fairly modest (Luostarinen et al. 1999). There is even a specific syndrome of epilepsy, cerebral calcifications and coeliac disease (Visakorpi et al. 1970, Gobbi et al. 1992), but for some reason it occurs almost exclusively in certain limited areas in Italy and Spain (Gobbi et al. 1992). In addition, some neuromuscular disorders and an early-onset dementia have been connected to coeliac disease, but their true prevalence remains to be elucidated (Cooke and Smith 1966, Hadjivassilou et al. 1997, Collin et al. 1991).

Coeliac patients may also suffer from severe psychiatric problems such as irritability, anxiety, depression and schizophrenia (Goldberg 1970, Kalaydjian et al. 2009). Particularly depression seems to be common in both adults and adolescents with coeliac disease (Hallert and Aström 1982, Pynnönen et al. 2004, Ludvigsson et al. 2007), although it has recently been suggested that depression is increased mainly in patients having some co-morbidity such as type 1 diabetes mellitus (Garud et al. 2009).

Coeliac disease may be a contributing factor in many gynaecological and obstetrical disorders such as infertility (Morris et al. 1970, Collin et al. 1996b), delayed menarche, amenorrhoea and early menopause (Ferguson et al. 1982, Smecuol et al. 1996). Furthermore, increased risks of miscarriages, prematurity, intrauterine growth retardation and low birth weight have been reported (Joske and Martin 1971, Ciacci et al. 1996, Gasbarrini et al. 2000, Martinelli et al. 2000). On the other hand, there are also controversial results showing no increase in either unexplained infertility or

pregnancy complications despite untreated coeliac disease (Kolho et al. 1999, Grego et al. 2004, Tata et al. 2005).

Liver abnormalities are common and may appear either with or without other clinical symptoms of coeliac disease, and even in children (Hagander et al. 1977, Bardella et al. 1995, Farre et al. 2002). The hepatic injury seems to be gluten-sensitive and its severity may vary from only slightly elevated transaminases to severe liver failure requiring transplantation (Hagander et al. 1977, Bardella et al. 1995, Kaukinen et al. 2002).

Other frequently observed extraintestinal manifestations of coeliac disease are permanent dental enamel defects (Aine et al. 1996), recurrent aphthous ulcerations (Ferguson et al. 1976), arthritis (Mäki et al. 1988b, Collin et al. 1992) and alopecia areata (Corazza et al. 1995a).

2.2.3 Malignancies

The most severe complications of coeliac disease are certain malignancies. Previously, when adult patients were rarely diagnosed and often inadequately treated, the risk particularly of non-Hodgkin lymphomas was considered to be substantial (Holmes et al. 1989). However, in recent studies the relative risk of these rather uncommon malignancies has been only about two to six times higher than in the healthy population (Collin et al. 1996a, Askling et al. 2002, West et al. 2004a, Viljamaa et al. 2006). Nevertheless, it is possible that long diagnostic delay or poor adherence to a gluten-free diet increases the risk of intestinal lymphomas (Freeman 2004), and especially so-called refractory coeliac disease seems to be a predisposing factor (Al-Toma et al. 2006a). Coeliac disease has also been connected to other malignancies such as oesophageal, large-intestine and liver cancers (Holmes et al. 1976, Askling et al. 2002, West et al. 2004a), but the findings are somewhat controversial (Viljamaa et al. 2006). For some unknown reason coeliac patients may have a reduced risk of lung and breast cancers (West et al. 2004a, Viljamaa et al. 2006).

2.2.4 Bone in coeliac disease

The association between bone disorders and coeliac disease has long been known (Salvesen and Boe 1953), but the formerly common osteomalacia is nowadays rare and osteoporosis is the most common complication at least in developed countries (Valdimarsson et al. 1994). Dual energy X-ray absorptiometry (DEXA) has shown that decreased BMD is common both in untreated children (Mora et al. 1993, Tau et al. 2006) and in adults with coeliac disease (Caraceni et al. 1988, Mazure et al. 1994, Valdimarsson et al. 1994, Corazza et al. 1995b). In addition, untreated run an approximately two- to four-fold risk of osteoporotic fractures compared with the healthy population (Vasquez et al. 2000, West et al. 2003a). The pathophysiological mechanisms underlying the bone loss involved are somewhat obscure, but it is evident that small-bowel mucosal damage reduces intestinal calcium and vitamin D absorption and thus leads to secondary hyperparathyroidism and bone resorption (Corazza et al. 1995b). This notwithstanding, osteoporosis can be present even while the villous structure is still morphologically normal (Mustalahti et al. 1999, Kaukinen et al. 2001), and it has been suggested that mucosal inflammation affects bone loss by disturbing the normal balance of bone remodelling (Taranta et al. 2004). Furthermore, antibodies against skeletal transglutaminase (TG) (Sugai et al. 2002) or osteoclastic regulator osteoprotegerin (Riches et al. 2009) may contribute to bone deterioration.

2.2.5 Coeliac disease and quality of life

The health-related quality of life comprises many physical, social and emotional dimensions, and includes an overall satisfaction with life and a general sense of well-being (Usai et al. 2002). Reproducible and validated methods should be used when quantifying the effect of a disease on the quality of life, and in coeliac disease research mostly generic questionnaires such as Psychological General Well-Being (PGWB) (Dupuy 1984) and the 36-item short-form (SF-36) (Ware and Sherbourne 1992) have been applied. In addition, most studies have been cross-sectional and conducted among adults having the classical intestinal form of coeliac disease (Häuser et al. 2006, Nachman et al. 2009).

In the majority of studies the quality of life has been decreased in untreated coeliac disease patients compared either with healthy controls or with the general population

(Johnston et al. 2004, Viljamaa et al. 2005a, Nachman et al. 2009). In contrast, the effect of a gluten-free diet on the quality of life is still somewhat unclear. In many studies coeliac disease patients have achieved normal quality of life while on a gluten-free diet (Lohiniemi et al. 2000, Johnston et al. 2004, Roos et al. 2006, Nachman et al. 2009), but there are also controversial results showing decreased quality of life despite treatment (Hallert et al. 1998, Addolorato et al. 2001, Häuser et al. 2006). Usually the severity of gastrointestinal symptoms has correlated with poorer quality of life (Usai et al. 2002, Johnston et al. 2004, Nachman 2009), but Mustalahti and associates (2002) have suggested that also asymptomatic coeliac patients benefit from a gluten-free diet. Furthermore, in one recent study the screen-detected patients have evinced normal quality of life while on a long-term gluten-free diet (Viljamaa et al. 2005a), which is important, since the dietary restrictions could also increase the burden of illness (Hallert et al. 2002).

Relatively few studies have assessed the quality of life in children and adolescents with coeliac disease. In a study by group under Kolsteren (2001) paediatric patients evinced quality of life comparable to that of general population, but recently the same group observed decreased quality of life when more age- and disease-specific questionnaires were used (van Doorn et al. 2008). In any case, those coeliac disease patients who have been diagnosed during childhood seem to achieve in adulthood a quality of life similar to that in the general population (Wagner et al. 2008).

2.3 Associated conditions and silent coeliac disease

Previously, when the high prevalence was unknown, coeliac disease was taken to be associated with numerous disorders, in most cases probably simply by coincidence. Nevertheless, in certain diseases an unusually high prevalence of coeliac disease has been regularly observed (Table 1). The best-known co-morbidities are type 1 diabetes mellitus (Visakorpi 1969), autoimmune thyroidal diseases (Kuitunen et al. 1971) and primary Sjögren´s syndrome (Pittman and Holub 1965). These associations are at least partly explained by shared genetic risks, but it is possible that other pathogenetic mechanisms are also involved. It is unclear whether the age at coeliac disease diagnosis or the dietary treatment has any effects on the development or severity of the co-morbidities (Ventura et al. 1999, Viljamaa et al. 2005b). Besides autoimmune

disorders, coeliac disease is overrepresented in some chromosomal abnormalities such as Down´s and Turner´s syndromes (Bentley 1975, Thatcher et al. 1973). In addition, coeliac disease is common in patients with selective IgA deficiency, which is clinically important as these subjects lack IgA-class antibodies (Savilahti et al. 1971, Meini et al. 1996).

Due to augmented serological screening, asymptomatic subjects with positive coeliac antibodies and a small-bowel mucosal villous atrophy are frequently found (Ferguson 1993, Vilppula et al. 2008). This is particularly true in patients belonging to some of the aforementioned risk groups and in the family members of coeliac patients (Mäki et al. 1991b). Although the natural history of patients having this so-called silent coeliac disease is poorly understood, evidence suggests that they may carry an increased risk of osteoporosis and bone fractures (Mazure et al. 1994, Mustalahti et al. 1999). Furthermore, the quality of life and BMD of asymptomatic patients may improve while on a gluten-free diet (Mustalahti et al. 1999, Mustalahti et al. 2002). However, in other studies even untreated asymptomatic patients have evinced normal quality of life (Johnston et al. 2004, Nachman et al. 2009). As screen-detected subjects also seem to have a low risk of intestinal lymphomas (Mearin et al. 2006) and often poor dietary compliance (Fabiani et al. 2000), more studies are needed to clarify whether all asymptomatic coeliac patients should be diagnosed.

Table 1. The prevalence of coeliac disease in subjects with associated disorders.

Condition and reference	Study population	Antibody positivity (%)	Coeliac disease (%)
Type 1 diabetes mellitus			
Mäki et al. 1984a	215 children	ARA 4.2	2.3
Hansen et al. 2001	106 children	AGA 18.3, EmA 9.6, TG2-abs 9,6	10.4
Thyroid disorders			
Collin et al. 1994	83 adults	AGA 18.1, ARA 4.8, EmA 4.8	4.8
Larizza et al. 2001	90 children	EmA 6.6	7.8
Sjögrens´s syndrome			
Iltanen et al. 1999a	34 adults	AGA 38.2, EmA 8.8	14.7
Szoroday et al. 2004	111 adults	EmA and TG2-abs 5.4	4.5
Addisons´s disease			
Myhre et al. 2003	76 children and adults	AGA 27.6, EmA 6.7, TG2-abs 6.7	7.8
Betterle et al. 2006	5 children, 105 adults	TG2-ab 3.7	2.8
Primary biliary cirrhosis			
Dickey et al. 1997	57 adults	EmA 10.5	7.0
Volta et al. 2002	173 adults	EmA 4.0 TG2-abs 4.0	4.0
Autoimmune hepatitis			
Volta et al. 1998	181 children and adults	AGA 13.8, EmA 4.4	2.8
Villalta et al. 2005	47 adults	EmA 6.4 TG2-abs 6.4	6.4
Autoimmune myocarditis			
Frustaci et al. 2002	187 adults	EmA 4.8, TG2-abs 7.0	4.8
Down´s syndrome			
Bonamico et al. 2001a	1110 children, 92 adults	AGA 21.5, EmA 5.4	4.6
Wouters et al. 2009	155 children	EmA 5.2, TG2-ab 5.2	4.5
Turner´s syndrome			
Ivarsson et al. 1999	87 children	AGA 14.9, EmA 4.6	4.6
Frost et al. 2009	256 adults	EmA 3.2	4.7
Selective IgA deficiency			
Meini 1996	65 children	AGA 24.6	7.7
IgA nephropathy			
Collin et al. 2002	223 children and adults	EmA 1.8, TG2-ab 3.6	3.6

ARA, antireticulin antibodies; AGA, antigliadin antibodies; EmA, endomysial antibodies, TG2-abs, transglutaminase 2 antibodies

3. SMALL-BOWEL MUCOSAL DAMAGE

The development of intestinal biopsy devices enabled the assessment of coeliac disease-associated small-bowel mucosal damage in a living person, and it was discovered that while in a healthy mucosa there are long finger-like villi and short crypts, characteristic of untreated coeliac disease are villous atrophy and crypt hyperplasia (Shiner 1957). The most seriously affected areas are usually the duodenum and the proximal part of jejunum, but the mucosal lesion may be patchy and variable along the whole length of the small intestine (MacDonald et al. 1964, Scott and Losowsky 1976). Although considered diagnostic, the villous atrophy is not pathognomic for coeliac disease, being equally possibly in disorders such as rotavirus infection, cow's milk allergy, autoimmune enteropathy, giardiasis, tuberculosis, Crohn´s disease and acquired immunodeficiency syndrome (Kuitunen et al. 1975, Green and Cellier 2007).

Besides morphological changes, there is an ongoing intestinal inflammation in untreated coeliac disease and the density of the mucosal cluster-design (CD) 3+ intraepithelial lymphocytes (IELs) is usually increased (Ferguson and Murray 1971, Kuitunen et al. 1982, Järvinen et al. 2003). Although most of these IELs express αβ+ T cell receptors, a finding more specific to coeliac disease is an abnormally high number of γδ+ IELs (Halstensen et al. 1989, Savilahti et al. 1990, Spencer et al. 1991). In addition, the density of other inflammatory cells such as mast cells and eosinophils is often increased in the lamina propria (Savilahti 1972). Another inflammatory marker of coeliac disease is an abnormal expression of human leukocyte antigen (HLA) DR antigens (Scott et al. 1981). While in untreated patients gluten-dependent HLA-DR expression can be seen in the epithelium and in the crypt enterocytes, in non-coeliac subjects there is no crypt expression and only mild staining of villous enterocytes is detectable (Scott et al. 1981, Arnaud-Battandier et al. 1986). However, all the aforementioned inflammatory changes are unspecific and may be present for example in cow's milk allergy, autoimmune diseases and parasitic infections (Ferguson and Murray 1971, Kuitunen et al. 1975, Kuitunen et al. 1982, Kakar et al. 2003). Even the γδ+ IELs, which are usually considered to be the most specific markers of coeliac disease, may be increased in other conditions and even without coeliac-type HLA-DQ2 or DQ8 (Spencer et al. 1991, Iltanen et al. 1999b, Kokkonen et al. 2000).

It is important to realize that the small-bowel mucosal deterioration in coeliac disease develops gradually from completely normal mucosa to increased intraepithelial lymphocytosis, then to hyperplasic crypts, and finally to villous atrophy with crypt hyperplasia (Figure 1). Depending on the degree of villous damage the mucosal lesion can be further classified into partial, subtotal and total villous atrophy (Kuitunen 1982, Marsh 1992). Furthermore, the villous height/crypt depth ratio (Vh/CrD) can be calculated, and a ratio <2.0 has usually been used to differentiate the coeliac lesion from normal mucosal morphology (Kuitunen et al. 1982).

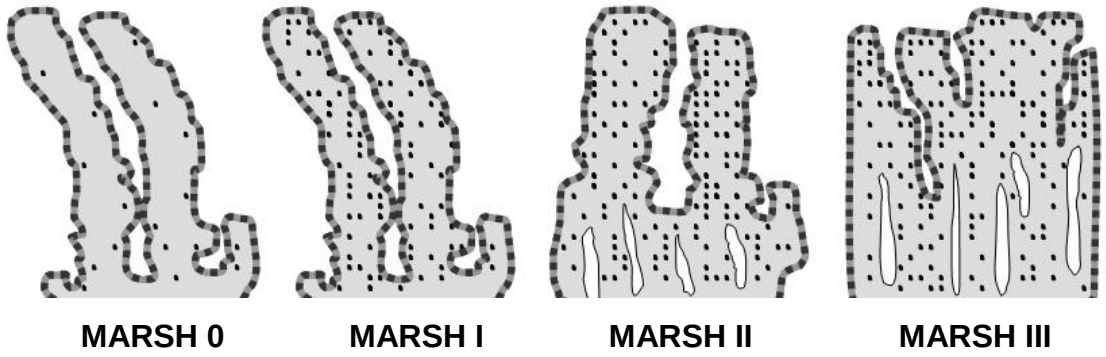

Figure 1. Gradual development of the small-bowel mucosal damage in coeliac disease. Adapted from Marsh (1992).

4. ANTIBODIES IN COELIAC DISEASE

In the early 1970s it was discovered that the sera of untreated coeliac patients contained antibodies against food-derived gluten peptides and against certain tissue structures (Seah et al. 1971, Carswell and Ferguson 1972) and it was soon realized that these antibodies could be used as non-invasive serological screening tools. Particularly when the enzyme-linked immunosorbent assay (ELISA) methods became available for measuring certain coeliac disease-associated antibodies, the diagnostic approach to the disorder changed completely (O´Farrelly et al. 1983, Sulkanen et al. 1998a).

4.1 Serum antigliadin antibodies

Serum antigliadin antibodies (AGA) were previously widely used in case-finding in coeliac disease (Hill et al. 2005). Unfortunately, AGA may be present in many disorders other than coeliac disease (Unsworth et al. 1983, Mäki 1995), and studies have shown heterogeneous sensitivities and specificities from only about 30% up to 100% (McMillan et al. 1991, Mäki et al. 1991, Vogelsand et al. 1995, Sulkanen et al. 1998a). Furthermore, by reason of the variable accuracy of the IgA- and IgG-class AGA among coeliac patients and the possibility of selective IgA deficiency, the antibodies are usually measured together (Savilahti et al. 1983). In view of this diversity of results, AGA are not currently recommended as the primary serological method for suspected coeliac disease, although they may still be valuable in children below two years of age (Hill et al. 2005, Maglio et al. 2010).

Interestingly, a necessary step in the pathogenesis of coeliac disease is a deamidation of gliadin peptides to form more specific epitopes to intestinal T-cells (Molberg 1998). The immune system may also form antibodies also against these deamidated gliadin peptides (Aleanzi et al. 2001), and several recent studies have shown that these antibodies have significantly higher diagnostic accuracy than the conventional AGA (Scwertz et al. 2004, Kaukinen et al. 2007a). Besides being accurate, antibodies to deamidated gliadin peptides might be the first serological markers of untreated coeliac disease, indicating that they could offer a promising method for the early detection of coeliac patients (Simell et al 2007, Liu et al. 2007).

4.2 Serum reticulin, endomysial and TG2 antibodies

The first autoantibodies in coeliac disease were discovered by Seah and associates (1971), who detected specific a antibody reaction against reticulin fibres in connective tissue. These R1-type antireticulin antibodies (ARA) could be measured in both IgA and IgG class by using indirect immonoflurescence on rodent tissues, but the IgA-class antibodies were later shown to be superior for untreated coeliac disease (Mäki et al. 1984b). The sensitivity of ARA has sometimes been rather low (Volta et al. 1991), but in most studies the specificity has been more than 90% and thus substantially higher than that of AGA (Seah et al. 1971, Mäki et al. 1984b, Hällström 1989, Kolho and Savilahti 1997). Due to this superior specificity, ARA were widely used even in the

late 1990s, but in 1983 a new autoantibody targeted against monkey oesophageal endomysium was discovered (Chorzelski et al. 1983). These endomysial antibodies (EmA) were shown to have sensitivity and specificity for coeliac disease similar to or even better than ARA (Table 2), but they became widely accepted after Ladinser and collegues (1994) observed that human umbilical cord could be used as a substitute for monkey oesophagus.

Despite the high accuracy of ARA and EmA it remained unclear which were the antigens they recognized until Dieterich and associates (1997) discovered that the autoantigen in monkey oesophagus smooth muscle cell endomysium was TG2. TG2 is ubiquitous enzyme catalyzing different transamidating and deamidating reactions, and also having a major role in the pathogenesis of coeliac disease (Molberg et al. 2000). Antibodies specific to TG2 (TG2-abs) could be measured and evinced high accuracy for coeliac disease comparable to EmA (Dieterich et al. 1998, Sulkanen et al. 1998a). A few years later Korponay-Szabo and associates (2000, 2003a) showed that ARA, EmA and TG2-abs are in fact structurally almost identical and that ARA were also targeted against TG2. As the TG2-abs can be measured by a practical ELISA method (Sulkanen et al. 1998a) they are often used, with human recombinant TG2 as an antigen, as the primary screening method for coeliac disease in clinical practice.

Although the introduction of EmA and TG2-abs brought a great advance in non-invasive case-finding in coeliac disease, they have certain limitations. When applied in a validated laboratory EmA shows excellent specificity, but the immunofluerescence method is laborious, time-consuming and somewhat subjective. In addition, the good accuracies of EmA and TG2-abs have been shown mainly in well-controlled research conditions and might be poorer in clinical settings (Rostami et al. 1999). Particularly TG2-abs have yielded relatively heterogeneous results depending on the laboratory or methods used (Hopper et al. 2007), and both antibodies are less reliable in children below two years of age (Mankai et al. 2005). Furthermore, in the case of selective IgA deficiency, the antibodies should be measured in IgG class (Sulkanen et al. 1998b, Korponay-Szabo et al. 2003b).

Table 2. Studies of the sensitivities and specificities of serum IgA-class endomysial (EmA) and tissue transglutaminase 2 (TG2-ab) antibodies for untreated coeliac disease

			EmA		TG2-ab	
Reference	Patients	Controls	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Hällström et al. 1989	14 children, 32 adults	24 children, 145 adults	93.5	99.3	ND	ND
Sulkanen et al. 1998a	136 children and adults	207 children and adults	92.6	99.5	94.9	93.7
Biagi et al. 1999	39 adults	61 adults	100	100	94.8	90.1
Bonamico et al. 2001b	62 children	56 children	95.2	98.2	90.3	100
Bürgin-Wolff et al. 2002	208 children and adults	157 children and adults	96.6	100	96.1	99.4
Tesei et al. 2003	250 adults	176 adults	85.6	100	90.0	94.9
Mankai et al. 2005	97 children, 46 adults	74 children and adults	95.8	100	86.0	95.9
Collin et al. 2005a	126 children and adults	106 children and adults	88.9	98.1	93.6	99.1
Volta et al. 2008	128 children and adults	134 adults	93.7	100	96.8	91.0
Raivio et al. 2008	139 children and adults	103 children and adults	99.3	100	98.6	99.0
Hopper et al. 2008	77 adults	1923 adults	87.0	98.0	90.9	90.9

ND, no data

4.3 Small-bowel mucosal autoantibody deposits

A few decades ago it was observed that the small-bowel mucosa of coeliac disease patients contained specific deposits of immunoglobulin A (IgA deposits) (Shiner and Ballard 1972, Kárpáti et al. 1988), which were subsequently shown to be targeted against extracellular TG2 (Korponay-Szabo et al. 2004). These deposits can be found in practically all patients having classical villous atrophy coeliac disease (Koskinen et al. 2008). Since the TG2-abs are also produced locally in the mucosa (Marzari et al. 2001), it seems that after formation the autoantibodies bind primarily to the extracellular TG2 and as a spilling effect enter into circulation (Korponay-Szabo et al. 2004). In fact, the antibodies can be detected in the mucosa even while not present in serum (Salmi et al. 2006a). This is important in that the sensitivity of EmA or TG2-abs is not always optimal and sometimes coeliac disease patients are missed due to false seronegativity. Interestingly, IgA deposits can be found even before villous atrophy develops (Kaukinen et al. 2005, Salmi et al. 2006b), and since the deposits also evince high specificity (Kaukinen et al. 2005, Tosco et al. 2008, Koskinen et al. 2008), they could be utilized in cases with strong clinical suspicion of coeliac disease but normal mucosal structure and negative autoantibodies. Nevertheless, the sensitivity of the deposits may be lower in children below two years of age (Maglio et al. 2010), and the need for special methods and frozen samples limits their utility in clinical settings.

4.4 Rapid tests

The coeliac autoantibodies can be measured only in specialized laboratories, which is expensive, time-consuming and even impossible in many developing countries. These problems have led to the development of new on-site tests in which high accuracy is combined with very short measurement time (Sorell et al. 2002). Particularly interesting is a new self-TG2-based point-of-care test, as it requires only a fingertip blood sample and evinces high accuracy for coeliac disease similar to the conventional EmA and TG2-abs (Korponay-Szabo et al. 2005, Raivio et al. 2006).

5. PATHOGENETIC ASPECTS OF COELIAC DISEASE

The development of coeliac disease is an end result of a complex interaction between genes, environmental factors and the immunological system of the small intestine. As in many complex diseases, the contribution of different predisposing and protecting factors in coeliac disease is difficult to assess, but rapid developments in the fields of genetics and immunology in the last few decades have enormously increased our understanding of the pathogenesis.

5.1 Genetics

The heritability of coeliac disease was shown by MacDonald and colleagues (1965) more than 40 years ago. The prevalence of the disorder among first-degree relatives is approximately 10-20% (Mäki 1991b, Greco 2002) and the high concordance rate of up to 90% between monozygotic twins further confirms its genetic nature (Hervonen et al. 2000, Greco et al. 2002). The association of coeliac disease with certain HLA molecules in chromosome region 6p21.3 was discovered in the early 1970s (Stokes et al. 1972) and was later defined to comprise specific alleles encoding HLA DQ2 and DQ8 molecules (Sollid et al. 1989). Lundin and associates (1993, 1994) demonstrated the causal connection of these haplotypes by showing that gluten activates HLA DQ2- and DQ8-restricted T-cells in the small intestine of coeliac disease patients. The observed correlation between the number of predisposing HLA-DQ alleles and the risk of coeliac disease gives further evidence for the role of these haplotypes in the pathogenesis (Vader et al. 2003).

More than 90% of coeliac patients have the HLA DQ2 (DQA1*0501/DQB1*0201) and almost all the rest the DQ8 (DQA1*0301/ DQB1*0302) haplotype (Sollid and Thorsby 1993, Karell et al. 2003). However, although the combined prevalence of HLA DQ2 and DQ8 in the population is approximately 30%, most individuals will never develop coeliac disease. Furthermore, the concordance of the disorder among HLA compatible siblings is only about 30% (Mearin et al. 1983). It has since been estimated that the HLA region contributes to less than half of the genetic risk for coeliac disease and numerous studies have sought to uncover potential non-HLA risk genes (van Heel et al. 2007, Hunt et al. 2008). Thus far results have been somewhat inconsistent and have varied between different populations, but an association with

certain specific chromosome regions, particularly 5q31-33, 2q33 and 19p13, has frequently been reported (Liu et al. 2002, Holopainen et al. 2004, Monsuur et al. 2005). These findings are supported by the biologically relevant candidate genes detected within these chromosome areas, for example CTLA-4, ICOS and MYO9B (Holopainen et al. 2004, Monsuur et al. 2005). In addition, three large-scale genome-wide association studies in coeliac disease have recently been conducted and as a result several new potential non-HLA loci have been identified. These new genetic risk regions are particularly interesting since many of them have been linked to type 1 diabetes and also to some other autoimmune diseases (van Heel et al. 2007, Hunt et al. 2008, Dubois et al. 2010). Nevertheless, it seems that the currently known non-HLA regions explain only a tiny fraction of the genetic background of coeliac disease (Dubois et al. 2010).

5.2 Environmental factors

5.2.1 Dietary factors

Dietary prolamins in wheat, barley and rye are the principal environmental trigger for coeliac disease. Wheat prolamins normally function as a storage protein in the grain and are composed of two different protein fractions, namely gliadins and glutenins. In addition, the barley and rye prolamins hordein and secalin contain structurally similar proteins and can also cause coeliac disease (Anand et al. 1978). The three-dimensional structure of gliadin can enhance the baking properties of dough and breeding has thus favoured cereals with a high gluten concentration, simultaneously increasing the risk of coeliac disease at population level (Guandalini 2008).

Besides being necessary triggering factor, the age at gluten introduction and its amount in the diet in infancy may influence the risk of coeliac disease, as demonstrated by a remarkable increase in the coeliac disease incidence in Sweden after new feeding recommendations in the 1980s (Ascher et al. 1993). It was later shown that children who developed coeliac disease consumed higher amounts of gluten than their healthy counterparts (Ivarsson et al. 2002). Although in the study in question the age at gluten introduction did not affect to coeliac disease risk, it has since

been suggested that the optimal age to introduce gluten would be between four to six months (Norris et al. 2005).

Whether breastfeeding or its duration affects to risk of coeliac disease is difficult to evaluate by reason of the obvious connection with gluten consumption. Epidemiological evidence suggests that breastfeeding might offer protection (Andersen and DiSant´Agnese 1953, Ivarsson et al. 2002), but there are results to the contrary and it is possible that prolonged breastfeeding only delays the onset of clinical symptoms (Akonbeng et al. 2006).

5.2.2 *Other environmental factors*

Certain microbes such as adeno- and rotaviruses have been suggested to increase the risk of coeliac disease (Kagnoff et al. 1987, Stene et al. 2006, Zanoni et al. 2006) and the seasonal pattern observed in the incidence supports the role of infections (Ivarsson et al. 2003). On the other hand, recurrent infections may have a protective role. The prevalence of coeliac disease and many other autoimmune diseases has increased rapidly in the past few decades (Bach et al. 2002, Lohi et al. 2007), suggesting that decreased exposure to microbiological agents in childhood predisposes the immune system to react against self-antigens (Strachan 1989, Plot et al. 2009). This theory was recently supported by Kondrashova and associates (2008), who observed a six-fold lower prevalence of coeliac disease in Russian Karelia than in Finland. Since people in these two areas have a similar genetic background and daily gluten consumption, the authors hypothesized that the disparity was caused by the lower standard of living and higher exposure to infections in Russia.

Certain other aspects of lifestyle and environment such as cigarette smoking and socioeconomic status may modulate the risk of developing coeliac disease (Austin et al. 2002, Ivarsson 2005). However, the evidence is mainly epidemiological and more studies are needed to assess the true causal implication of these environmental factors.

5.3 Pathogenetic mechanisms

The toxicity of wheat in coeliac disease is connected mainly to certain specific gliadin peptides (Sollid 2000). Characteristic of gliadin is the high concentration of the amino

acids proline and glutamine, which enable immunogenic polypeptides to survive intestinal degradation (Frazer et al. 1959, Shan et al. 2002). Before immunological reactions can be initiated the gliadin peptides must penetrate the intestinal epithelium, which under normal conditions is highly resistant. However, in untreated coeliac disease an increased permeability can be observed, although the mechanisms behind this are still somewhat obscure (Madara et al. 1980, Fasano et al. 2000). It has been recently observed that in coeliac disease gluten induces a release of a certain prehaptoglobulin 2 precursor called zonulin (Fasano et al. 2000, Tripathi et al. 2009). Zonulin is a regulator of the epithelial tight junctions and after the binding of gliadin to the chemokine receptor CXCR3 zonulin may increase the paracellular permeability (Lammers et al. 2008). In addition, the gliadin peptides could pass the epithelium with the help of dendritic cells (Rescigno et al. 2001) or by a transcellular pathway through enterocytes (Zimmer et al. 1995, Matysiak-Budnik et al. 2003, Schumann et al. 2008).

Once the immunogenic peptides reach the submucosal layer, they can activate adaptive immunological reactions. The process starts when gluten peptides are presented to specific CD4+ T cells by the HLA DQ2 or DQ8 molecules of antigen-presenting cells (Lundin et al. 1993 and 1994). Intact peptides lack negatively charged amino acid residues and thus have a low affinity for HLA DQ molecules (van de Wal et al. 1996). However, the high concentration of glutamine in gluten makes it an excellent substrate to TG2, which specifically deamidates the glutamine residues to negatively charged glutamate, thus enabling the formation of HLA DQ-gliadin complexes and subsequent CD4+ T cell responses (Molberg et al. 1998, van de Wal et al. 1998). The activation of T cells leads to the production of cytokine interferon γ (Nilsen et al. 1998) which, alongside other inflammatory mediators such as tumour necrosis factor α, can induce crypt hyperplasia and exert direct cytotoxicity on epithelial cells (Bajaj-Elliot et al. 1998, Deem et al. 1991). Furthermore, the increased release of different metalloproteinases leads to deterioration of the small-bowel mucosal structure (Daum et al. 1999).

The important role of the innate immune system in the pathogenesis of coeliac disease has recently been recognized. Studies have shown that gluten triggers immunological responses independently of adaptive T cell reactions and that interleukin 15 (IL-15) is the most important mediator of these responses (Jabri et al. 2000, Maiuri et al. 2003). In the small-bowel mucosa innate reactions can increase the density of IELs, and subsequently cause intestinal epithelial cell lysis and mucosal

damage through the NKG2D-MIC pathway (Hüe et al. 2004). These observations suggest that both innate and the adaptive immunology are required for the development of coeliac disease, this perhaps partly explaining why only a minority of those who have the HLA DQ2 or DQ8 genotype will eventually suffer from the disorder (Maiuri et al. 2003).

The presence of TG2-abs is a hallmark of coeliac disease (Dietrich et al. 2007), but their significance in the pathogenesis has remained obscure. These antibodies are formed in the intestinal mucosa (Marzari et al. 2001), but in contrast to gliadin-specific T cells (Molberg et al. 1997), intestinal TG2-specific T cells have not been detected and it is still unclear how the TG2-abs are produced (Lindfors et al. 2009). As a possible explanation, a so-called hapten-carrier mechanism has been proposed (Mäki 1994, Sollid et al. 1997). In this model the gluten peptides and TG2 are presented together to the gluten-specific CD4+ T cells, which then help B cells to produce TG2-specific antibodies. In addition, some forms of molecular mimicry may participate in this antibody production (Kagnoff et al. 1987, Zanoni et al. 2006, Korponay-Szabo et al. 2008).

Once formed, the TG2 antibodies may evince biological activity. Halttunen and Mäki (1999) have shown that IgA from untreated coeliac patients inhibits the differentiation of intestinal epithelial cells in vitro. Furthermore, the antibodies can reduce mesenchymal cell motility and increase matrix degeneration (Barone et al. 2007), disturb growth of the villous structures by inhibiting angiogenesis (Myrsky et al. 2008), increase epithelial permeability (Zanoni et al. 2006) and modulate the transamidating activity of TG2 (Esposito et al. 2002). After entering the circulation the antibodies might also contribute to the extraintestinal manifestations by depositing in different tissues (Korponay-Szabo et al. 2004). Such autoantibody deposits have been detected around blood vessels of the liver in subjects with severe liver disease (Korponay-Szabó et al. 2004) and in the brain of gluten ataxia patients (Hadjivassiliou et al. 2008a). Furthermore, in a mouse model antibodies from an untreated coeliac patient were found deposited around the cerebellar blood vessels and caused ataxia-like symptoms (Boscolo et al. 2007). Interestingly, patients with dermatitis herpetiformis have autoantibodies targeted against TG3 (Sardy et al. 2003) and those with gluten ataxia against TG6 (Hadjivassiliou et al. 2008b), indicating that the extraintestinal manifestations could be determined by the type of TG against which the antibodies are targeted (Lindfors et al. 2009).

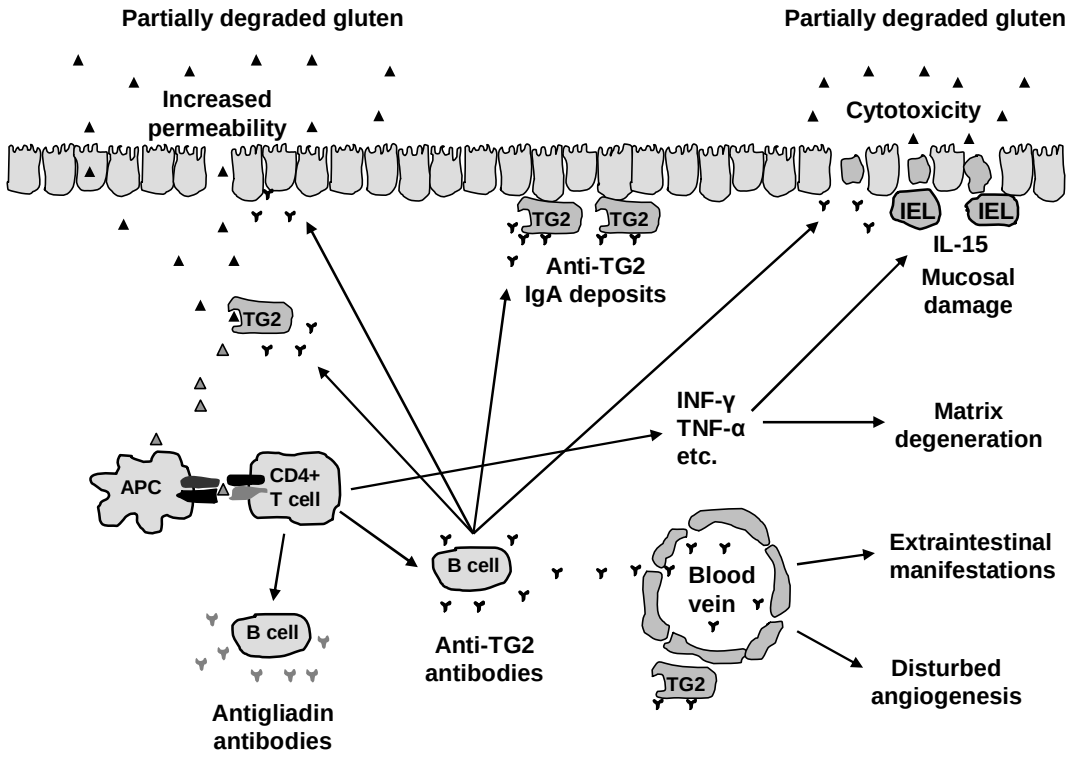

Figure 2. Pathogenetic mechanisms in coeliac disease. Incompletely digested gluten peptides pass the intestinal epithelium either paracellularly after increased zonulin release or through the enterocytes and are deamidated by transglutaminase 2 (TG2). The deamidated peptides are presented to T-cells by the antigen-presenting cells (APC), and subsequently produce proinflammatory cytokines such as interferon γ (INF-γ) and tumour necrosis factor α (TNF-α), which are detrimental to mucosal integrity. Other gluten peptides can be directly cytotoxic and activate innate immunity via interleukin 15 (IL-15). The gluten-stimulated T-cells also help B-cells to produce TG2-targeted autoantibodies, which may increase epithelial permeability, disturb angiogenesis and modulate the deamidating activity of TG2. In addition, the circulatory autoantibodies may participate in the extraintestinal manifestations of coeliac disease.

6. EPIDEMIOLOGY OF COELIAC DISEASE

Previously, when the extraintestinal and silent forms were largely unknown, coeliac disease was considered to be mainly a rare disease of childhood (Davidson and Fountain 1950). Even in the 1980s the prevalence was estimated to be about 0.1% and the incidence was actually thought to be decreasing (Stevens et al. 1987). It was, however, soon realized that the clinical presentation had become milder and had shifted to older age groups (Logan et al. 1983, Mäki et al. 1988a). Recent population-based screening studies have shown the prevalence of coeliac disease to be approximately 1% in both children (Fasano et al. 2003, Hoffenberg et al. 2003, Mäki et al. 2003, Korponay-Szabo et al. 2007) and adults (Fasano et al. 2003, West et al. 2003). The prevalence also appears to increase by age, as shown by the significantly higher seropositivity (up to 2.7%) in the elderly population (Vilppula et al. 2009). It is of note that not only the number of cases found but also the true prevalence of coeliac disease has increased. In a study by a group under Lohi (2007), coeliac seropositivity had almost doubled from one to two percent in the Finnish population during the past two decades, and a similar trend was recently observed in the United States (Rubio-Tapia et al. 2009). It is possible that changes in our microbiological environment are behind this phenomenon (Section 5.2.2).

There are substantial differences in the geographical distribution of coeliac disease. For example, the disorder is very rare in Asian countries like China and Japan, whereas so far the highest seroprevalence, 5.6%, has been detected among Saharawi children in Northern Africa (Catassi et al. 1999). These disparities are probably related to variations in genetic predisposition and the amount of gluten in the daily diet. In addition, an extraordinarily high prevalence of up to 3% has been revealed in children born during the Swedish epidemic of coeliac disease (Myléus et al. 2009; see Section 5.2).

7. TREATMENT OF COELIAC DISEASE

7.1 Dietary treatment

Strict avoidance of wheat, barley and rye is the cornerstone of treatment in all forms of coeliac disease. The safety of oats has been questioned since it is taxonomically related to the prohibited cereals, but prospective studies have shown that oats is safe for the majority of patients (Janatuinen et al. 1995, Högberg et al. 2004, Holm et al. 2006). Nevertheless, in some case reports oats has caused small-bowel mucosal damage and thus might not be suitable for all coeliac patients (Lundin et al. 2003). Another question has been whether the cereals in question should be naturally gluten-free, but several studies have shown that industrially purified wheat starch products which in theory might contain trace amounts of gluten are also safe for coeliac patients (Kaukinen et al. 1999, Lohiniemi et al. 2000, Peräaho et al. 2003).

The initiation of a gluten-free diet usually alleviates gastrointestinal symptoms within days and the small-bowel mucosal deterioration also begins to improve rapidly, although the complete normalization of the mucosa may take several years (Yardley et al. 1962, Grefte et al. 1988). In addition, most of the malabsorptive symptoms disappear on a gluten-free diet even without supplementary medication (Annibale et al. 2001). Dietary treatment is also beneficial in the case of extraintestinal manifestations and complications of coeliac disease. It may improve ataxia (Pellechia et al. 1995, Hadjivassilou et al. 2008) and peripheral neuropathy (Kaplan et al. 1988), reduce epileptic seizures (Hernández et al. 1998), alleviate depression (Pynnönen et al. 2005) and improve the quality of life (Hallert 1998). In addition, gynaecological and obstetric problems (Smecuol et al. 1996), arthritis (Mäki et al. 1988b, Collin et al. 1992) and liver dysfunction (Hagander et al. 1977, Bardella et al. 1995, Kaukinen et al. 2002) may disappear during dietary treatment. A gluten-free diet also seems to reduce both the risk of malignant diseases (Holmes et al. 1989, Collin et al. 1996a) and mortality in coeliac patients (Corrao et al. 2001). Decreased BMD usually increases while on diet (Mora et al. 1993, Valdimarsson et al. 1994, Mustalahti 1999, Tau et al. 2006), but to ensure normal bone maturation the treatment should be started as early as possible (Mora et al. 1999, Tau et al. 2006). Early-initiated treatment also enables successful catch-up growth in children with coeliac disease (Barr et al. 1966). Although a gluten-free diet is the treatment of choice in dermatitis herpetiformis (Fry

et al. 1973), sufferers seem to be particularly sensitive to trace amounts of gluten, and additive anti-inflammatory dapsone medication is often required for the first few years following the diagnosis (Kruizinga and Hamminga 1953, Reunala 2001).

It must be emphasized that despite all the aforementioned benefits, a life-long gluten-free diet is expensive and difficult to maintain, and may involve social restrictions (Hallert et al. 2002a, Lee et al. 2007). In addition, the diet may induce an undesirable intake of fat and sugar, leading to detrimental weight gain (Mariani et al. 1998, Dickey and Kearney 2006), and there is also evidence of poor vitamin status after a long-term gluten-free diet (Hallert et al. 2002b). Furthermore, long-term compliance with the gluten-free diet may be rather poor (Fabiani et al. 2000, Högberg et al. 2003, Whitaker et al. 2009), suggesting that there is a need for other treatment options.

7.2 New treatment options

Several alternative means of treating coeliac disease are emerging and some are already on clinical trial (Sollid and Khosla 2005). Unfortunately, a well-defined animal model of coeliac disease is lacking and thus potential new approaches can be tested only in cell and tissue models before human trials are launched.

Eradication of the toxic gliadin sequences from cereals by genetic engineering could prevent coeliac disease at population level (Vader et al. 2003), but at present technical and ethical problems restrict genetic modification of food products. Another preventive option could be degradation of the immunogenic polypeptides by means of specific endoproteolytic enzymes, either during baking or while in the stomach (Hausch et al. 2002, Piper et al. 2004, Stenman et al. 2009). Many of these enzymes have the benefit of being already used in the food industry and thus possessing a well-defined safety profile (Sollid and Khosla 2005). Finally, the penetration of the toxic peptides to the intestinal epithelium could be inhibited by using specific zonulin antagonists (Paterson et al. 2007).

Even after the toxic gliadin has entered the body the immunological reaction could be blocked at several points. Experimental evidence suggests that inhibition of TG2 may reduce gluten-induced T cell activation (Molberg et al. 2001, Maiuri et al. 2005). In addition, a number of other inhibitory agents such as HLA-DQ2 molecule blockers

and IL-10 or IL-15 agonists have been proposed (Sollid and Khosla 2005). Finally, the novel idea has been introduced of using peptide-based immunotherapy to tolerize patients to the toxic gliadin epitopes (Camarca et al. 2009).

7.3 Refractory coeliac disease

Occasionally coeliac patients fail to achieve a clinical and histological response despite treatment with a gluten-free diet. In these cases the treatment should be revised by a trained dietitian, since some inadvertent gluten intake may have remained. If no dietary failures are observed, the original diagnosis should be re-assessed. The small-bowel mucosal villous atrophy could also be caused by disorders other than coeliac disease (Chapter 3), but the presence of coeliac autoantibodies in the serum or in the small-bowel mucosa and the pertinent HLA type are strongly suggestive of correct diagnosis. If all other reasons for the treatment failure are excluded, the condition is defined as refractory coeliac disease (Trier et al. 1978).

Depending on the immunophenotype of the IELs, refractory coeliac disease can be divided into subtypes 1 and 2. Both subtypes are virtually non-existent in children, indicating that a long-term exposure to gluten is needed for their development (Al-Toma et al. 2007a). In addition, homozygosity for the HLA-DQ2 genotype or the presence of a specific MYO9B gene region are significant risk factors (Al-Toma et al. 2006a, Wolters et al. 2007). Patients with either type 1 or 2 refractory coeliac disease usually suffer from typical coeliac-type gastrointestinal symptoms and evince persistent small-bowel mucosal villous atrophy (Al-Toma et al. 2007a). However, while type 1 is a fairly benign disorder, in type 2 disease the immunophenotype of IELs is abnormal, showing clonal T cell receptor gene rearrangements and loss of T cell antigens, and it is a severe disease with high mortality (Cellier et al. 1998). In type 1 disease prednisolone and azatioprine are often effective (Goerres et al. 2003). In contrast, although intensive chemotherapy and immunomodulators may be helpful, the prognosis in type 2 disease is usually rather poor (Al-Toma et al. 2006b, Turner at al. 2005). If all other treatments have failed, autologous stem cell transplantation might be considered (Al-Toma et al. 2007b).

8. DIAGNOSTIC CRITERIA AND NATURAL HISTORY OF COELIAC DISEASE

8.1 Current diagnostic criteria

The first diagnostic criteria for coeliac disease were established by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) in 1970 (Meeuvisse 1970). In these criteria the diagnosis was based on three separate small-bowel biopsies showing an initial mucosal villous atrophy, then histological remission while on a gluten-free diet and finally mucosal relapse within two years on a gluten challenge. These criteria were revised in 1990 and it was stated that in symptomatic children at least partial small-bowel mucosal villous atrophy, together with a good clinical response to treatment, is sufficient for the diagnosis (Walker-Smith et al. 1990). However, in asymptomatic patients histological recovery should be demonstrated, and in unclear cases an additional gluten challenge is still recommended. The presence of coeliac antibodies is not necessary but gives further evidence for the diagnosis. These criteria are still widely used in all age groups, but in adults a second small-bowel biopsy is usually recommended regardless of the initial presentation (United European Gastroenterology 2001, Hill et al. 2005). The diagnosis of dermatitis herpetiformis is based on the demonstration of granular IgA deposits in the dermal papillae of the skin close to the active lesion (van der Meer 1969).

8.2 Early developing coeliac disease

There is mounting evidence to indicate that small-bowel mucosal villous atrophy with crypt hyperplasia represents only the end-point in the wide clinical spectrum of coeliac disease. The intestinal damage evolves gradually from completely normal mucosa to overt villous atrophy (Marsh 1992) and this deterioration may take years or even decades to develop despite continuous gluten consumption (Egan-Mitchell et al. 1981, Mäki et al. 1990, Collin et al. 1993, Lähdeaho et al. 2005). Furthermore, the severity of the clinical symptoms and the degree of mucosal villous atrophy do not necessary correlate (Kaukinen et al. 2001, Esteve et al. 2006, Murray et al. 2008). The well-defined concept of latent coeliac disease describes patients who have initially had

normal small-bowel mucosal structure but who have later developed villous atrophy while on a gluten-containing diet (Ferguson et al. 1993). A number of studies of latent coeliac disease are shown in Table 3, and it can be seen that in many cases patients have evinced clinical symptoms and signs of small-bowel mucosal inflammation while the villous structure was still normal. In consequence of the persisting symptoms and fear of possible complications, patients are often willing to start treatment despite the lack of a definite histological diagnosis. Table 4 shows studies in which an experimental gluten-free diet has been initiated without the demonstration of villous atrophy, and it can be seen that in many cases there has been beneficial serological and clinical response to early treatment, and also that the early mucosal changes have been alleviated.

Such results suggest that the current diagnostic criteria requiring small-bowel mucosal villous atrophy and crypt hyperplasia are insufficient to cover all the variable presentations of the coeliac-type disorders. Interestingly, there is already a well-defined model for coeliac disease without villous atrophy. Patients with dermatitis herpetiformis usually show signs of small-bowel mucosal damage indistinguishable from that in intestinal disease (Marks et al. 1966), and although in almost one third of cases there are only non-atrophic inflammatory changes in the mucosa, treatment with a gluten-free diet is still indicated (Savilahti et al. 1992, Reunala 2001). Similarly, patients with gluten ataxia may have positive serum coeliac antibodies, the appropriate HLA-type and even TG2-specific IgA deposits in the intestinal mucosa, but small-bowel villous atrophy is present in only a minority of these gluten-sensitive patients (Hadjivassiliou et al. 2008a). Nevertheless, intolerance to gluten-containing cereals and increased IELs are too unspecific for a definitive diagnosis of coeliac disease (Kaukinen et al. 2000, Kuitunen et al. 1982, Järvinen et al. 2003, Salmi et al. 2006) and more reliable markers for an early developing disorder are needed.

The presence of either the HLA DQ2 or DQ8 genotype is practically a requirement for coeliac disease, but is an unspecific finding and can be used only as an exclusive method before further investigations (Karell et al. 2003, Esteve et al. 2006). In contrast, an increased mucosal γδ+ IEL count is a much more specific marker, and may also be detectable before villous atrophy develops (Mäki et al. 1991a, Kaukinen et al. 1998, Iltanen et al. 1999c, Järvinen et al. 2004). However, even these cells may be elevated in other conditions (Spencer et al. 1991, Iltanen et al. 1999b, Järvinen et al. 2003), and the need for frozen sample and special methods limits their usefulness in

clinical practice. As an improvement to the classical morphometric methods requiring only paraffin sections, determination of villous tip IELs has been shown to evince specificity similar to that of γδ+ IELs, but further studies are needed to clarify the role of this method in diagnosis (Järvinen et al. 2004, Salmi et al. 2010). As a conclusion, it is obvious that all the aforementioned methods are insufficient for a definitive diagnosis of early-developing coeliac disease.

At present EmA and TG2-abs are frequently used as a first-line screening method in cases of coeliac disease suspicion before any histological investigation. As a result of intensified serological screening, subjects with positive antibodies but normal small-bowel mucosal villi are increasingly found (Mäki et al. 2003, Fasano et al. 2003, Ludvigsson et al. 2009). Although these subjects are usually considered to represent false positivity, it can be seen from the Table 3 that in many cases positive antibodies have actually predicted forthcoming villous atrophy. In fact, the appearance of these antibodies would appear to constitute very early phenomenon in the pathogenesis of coeliac disease, to be followed only later followed by the typical histological changes and clinical presentation (Korponay-Szabo et al. 2004, Simell et al. 2007 and 2010). Since particularly EmA and TG2-abs also have high specificity (Table 2), their presence before villous atrophy develops could offer a promising basis for early identification of the disorder. Nevertheless, thus far no controlled follow-up study has been carried out in subjects with positive coeliac antibodies but normal villous structure, and there is no consensus as to either their diagnosis or treatment with a gluten-free diet (Hill et al. 2005).

Table 3. Studies involving patients who had normal small-bowel mucosal stucture at baseline but who later developed villous atrophy and crypt hyperplasia while on a gluten-containing diet.

Reference	Total cases followed	Baseline symptoms in those who developed coeliac disease	Serum antibodies at baseline	Mucosal findings at baseline	Final diagnosis n (%)	Follow-up time median (range)
Weinstein 1974	2 adults	asymptomatic	ND	structurally normal	2 (100)	4 and 8 months
Egan-Mitchell et al. 1981	1 child	abdominal, poor growth	ND	structurally normal	1 (100)	1 year
Mäki et al. 1990	3 children and 1 adult	2 malabsorption, 1 extraintestinal, 1 asymptomatic	1/4 ARA and EmA	structurally normal, 1 had incresed IELs	4 (100)	5.7 (2.6-9) years
Mäki et al. 1991a	1 adult	asymptomatic	ARA	increased CD3+ and γδ+ IELs	1 (100)	2 years
Mäki et al. 1991b	7 children and adults	ND	7/7 ARA or EmA	structurally normal	3 (43) (3 had ARA at baseline)	3 years in all
Collin et al. 1993	25 adults	ND	6/25 ARA 22/25 AGA	structurally normal	7 (28) (6 had ARA at baseline)	3 (1-5) years
Troncone 1995	ND, children	3 abdominal, 4 poor growth, 2 extraintestinal, 3 asymptomatic	ND	structurally normal	14 (ND) (2 had EmA at baseline)	1.8 (1-10) years
Corazza et al. 1996	ND, adults	1 abdominal, 1 malabsorption, 1 asymptomatic	ND	structurally normal	3 (ND) (2 had EmA at baseline)	3 (0.2-13) years
Kaukinen et al. 1998	12 adults	3 abdominal, 1 weight loss, 1 anaemia	5/12 ARA, 8/12 AGA	increased CD3+, αβ+ and γδ+ IELs	5 (42) (2 had ARA at baseline)	ND (0.3-1.5) years
Iltanen et al. 1999b	18 children	ND	18/18 ARA	increased CD3+, αβ+ and γδ+ IELs	4 (22) (all had ARA at baseline)	ND (1.5-4.5) years

Iltanen et al. 1999c	ND, children	5 abdominal, 3 poor growth, 1 asymptomatic	ND	Marsh 0-I	9 (ND) (8 had EmA at baseline)	ND (0.8-4.5) years
Murray et al. 2001	14 adults	4 anaemia	3/9 EmA, 3/9 AGA	Marsh I-II	4 (29) (3 had EmA at baseline)	4 (3-5) years
Sbarbati et al. 2003	6 children and 1 adult	1 abdominal, 1 anaemia, 2 poor growth, 3 asymptomatic	7/7 EmA, 5/7 AGA	structurally normal	2 (40) (2 had EmA at baseline)	1 year in both
Järvinen et al. 2004	ND, adults	12 abdominal, 1 anaemia, 4 asymptomatic	ND	Marsh 0-I	17 (ND) (12 had EmA at baseline)	ND
Lähdeaho et al. 2005	148 children	ND	3/148 EmA, 5/148 AGA	structurally normal	6 (4) (3 had EmA at baseline)	10 (0.5-21) years
Dickey et al. 2005	8 adults	symptomatic	8/8 EmA	Marsh 0-I	6 (75) (all had EmA at baseline)	1.5 (1-6) years
Salmi et al. 2006b	47 adults	8 abdominal, 5 extraintestinal, 4 asymptomatic	ND	Marsh 0-I 14/15 IgA deposits	17 (36) (13 had ARA or EmA at baseline)	2.2 (0.2-7.4) years
Grodzinsky et al. 2008	19 children	ND	19/19 EmA	structurally normal	11 (58) (all had EmA at baseline)	ND
Mohamed et al. 2008	14 adults	3 abdominal, 1 anaemia, 1 extraintestinal, 1 weight loss	14/14 EmA 5/14 TG2-abs	increased CD2+, 3+, 7+, 8+ and 69+ IELs	6 (43) (all had EmA at baseline)	6.5 (4-12) years
Koskinen et al. 2008	ND, children and adults	17 abdominal, 5 extraintestinal, 2 malabsorption, 4 asymptomatic	ND	13/21 increased IELs, 25/26 IgA deposits	28 (ND) (19 had ARA or EmA+ at baseline)	1.7 (0.2-7.4) years

ND, no data; ARA, antireticulin antibodies; AGA, antigliadin antibodies; EmA, endomysial antibodies; TG2-abs, tissues transglutaminase 2 antibodies; IELs, intraepithelial lymphocytes

Table 4. Studies involving patients who were placed on experimental gluten-free diet despite structurally normal small-bowel mucosal villi.

		Baseline			After gluten-free diet		
Reference	Patients	Primary symptoms	Serum antibodies	Mucosal Histology	Symptoms	Serum antibodies	Mucosal Histology
Cooper et al. 1980	17 adults	17 abdominal	ND	increased IELs	alleviated in 9/17	ND	improved in 8/12
Arranz and Ferguson 1993	9 adults	8 abdominal, 1 extraintestinal	ND	5/9 increased IELs	alleviated in 7/9	ND	improved in 3/5
Picarelli et al. 1996a	10 adults	7 abdominal, 3 extraintestinal	10/10 EmA, 7/10 AGA	4/10 increased IELs	alleviated in all	decreased in all	improved in 4/4
Kaukinen et al. 2001	10 adults	7 abdominal, 2 weight loss, 1 extraintestinal	8/10 EmA, 9/10 TG2-abs	Marsh I-II	alleviated in all	decreased in 9/9	improved in all
Goldstein and Underhill 2001	10 adults	symptomatic	8/10 EmA, 5/10 AGA	structurally normal	alleviated in all	ND	ND
Wahnscaffe et al. 2001	26 adults	26 abdominal	26/26 negative	ND	alleviated in 17/26	ND	improved in 18/26
Tursi and Brandimante 2003	23 adults	4 abdominal, 2 extraintestinal	3/23 EmA, 6/23 TG2-abs	Marsh I-II	alleviated in all	decreased in 5/6	improved in 5/6
Kakar et al. 2003	4 adults	3 abdominal, 1 anaemia	3/3 EmA, 1/3 TG2-abs	increased IELs	alleviated in all	ND	ND
Järvinen et al. 2004	20 adults	13 abdominal, 1 anaemia, 1 extraintestinal, 5 asymptomatic	18/20 EmA	14/20 increased CD3+ and 19/20 γδ+ IELs	alleviated in all	decreased in 17/17	improved in 17/17

Paparo et al. 2005	6 children	4 symptomatic	6/6 EmA	increased CD3+ and γδ+ IELs	alleviated in all	decreased in all	ND
Kaukinen et al. 2005	20 adults	18 abdominal, 1 anaemia, 1 asymptomatic	2/20 EmA, 5/20 TG2-abs	increased γδ+ IELs, 4/20 IgA deposits	ND	ND	IgA deposits; lower intensity in 3/4
Dickey et al. 2005	27 adults	symptomatic	27/27 EmA	Marsh 0-I	alleviated in 26/27	decreased in 26/27	ND
Koskinen et al. 2008	20 adults	17 abdominal, 1 anaemia, 2 extraintestinal	14/19 EmA or ARA	18/20 increased IELs, 20/20 IgA deposits	alleviated in 18/20	decreased in 12/13	improved in 13/18, 2 ND
Ludvigsson et al. 2009	30 children and adults	abdominal symptoms in most	17/30 EmA, 8/30 TG2-abs	structurally normal	alleviated in15/30	ND	ND

IELs, intraepithelial lymphocytes; EmA, endomysial antibodies; AGA, antigliadin antibodies; TG2-abs; tissue transglutaminase 2 antibodies; ARA, antireticulin antibodies; IgA deposits, tissue transglutaminase 2-targeted autoantibody deposits; ND, no data

THE PRESENT STUDY

1. AIMS

With increasing knowledge of the variable phenotypes of coeliac disease it has come to be recognized that small-bowel villous atrophy and crypt hyperplasia represent only one of the many forms of the disorder. Nonetheless, the current diagnostic criteria for coeliac disease are still based on the demonstration of these particular manifestations. The main objective in the present study was to establish whether EmA-positive subjects with normal small-bowel mucosal morphology suffer from a coeliac-type disorder similar to that in patients already evincing diagnostic villous atrophy and crypt hyperplasia.

The specific aims were:

1. Compare clinical, serological and histological markers and complications related to coeliac disease in EmA-positive subjects with normal small-bowel mucosal structure to those found in individuals with overt villous atrophy, and to non-coeliac controls (**I-III**).

2. Establish whether early dietary treatment would be beneficial regardless of the small-bowel mucosal morphology by dividing EmA-positive subjects either to continue on a gluten-containing diet or to start an intervention with a gluten-free diet (**I-III**).

3. Establish whether the intestinal and extraintestinal forms of coeliac disease can fluctuate over time in the same patient on a gluten-containing diet (**IV**).

2. PATIENTS

2.1 Prospective studies (I-III)

All participants underwent clinical and serological evaluations and upper gastrointestinal endoscopy in the Departments of Gastroenterology and Alimentary Track Surgery (**I-II**) or Paediatrics (**III**) in Tampere University Hospital. Thereafter, EmA-positive subjects continued in the studies and were further classified according to the small-bowel mucosal morphology. The participants who had normal or non-coeliac mucosal villous structure (Marsh 0-II) comprised the study groups, whereas those with villous atrophy (Marsh III) comprised the coeliac disease control groups. The primary reason for coeliac disease suspicion, the duration of symptoms, past medical history and family history of coeliac disease were recorded in all cases at baseline.

Study **I** was a randomized trial. Altogether 145 adults were remitted to hospital from primary health care due to clinical suspicion of coeliac disease. Ten patients declined to participate and three cases were excluded by the presence of dermatitis herpetiformis. None was on immunosuppressive medication. At baseline, extensive clinical, serological and histological evaluations were carried out, thereafter EmA positive subjects were classified into normal villi (Marsh 0-II) and villous atrophy (Marsh III) groups as stated above. Next, patients with normal villous structure were randomized either to receive normal gluten-containing diet or to start on an experimental gluten-free diet. After one year the baseline investigations were repeated in all the EmA-positive cases. Subsequently, a gluten-free diet was also offered to those subjects who had been on a gluten-containing diet and a second follow-up visit was arranged after another year (Figure 1 in original publication **I**). Altogether 34 EmA-negative adults who were investigated because of dyspepsia or heartburn were used as non-coeliac controls for histological and serological evaluations. None of these subjects had a family history of coeliac disease.

Study **II** was an unrandomized trial. Collection of data began during Study **I** and the patients were to some extent the same with like inclusion criteria. Seven subjects declined to participate and three cases with dermatitis herpetiformis were excluded. None was using either immunosuppressive or antidepressive medication. Again, EmA-positive patients were classified into the normal villi (Marsh 0-II) and villous atrophy (Marsh III) groups; all subjects started a gluten-free diet. However, as 10 subjects in

the study group belonged in the randomization protocol of Study **I**, they remained on a gluten-containing diet for an additional year before the gluten-free diet was initiated (Figure 1 in original publication **II**). Alongside the serological and histological evaluations, self-rated gastrointestinal symptoms and health-related quality of life as well as BMD and BMI were measured both at baseline and after one year on a gluten-free diet. The non-coeliac controls for gastrointestinal symptom and health-related quality of life comparisons were obtained from the Finnish Coeliac Society and comprised a total of 110 adults who were friends or neighbours of coeliac patients and had no coeliac disease suspicion.

The cohort in Study **III** comprised 59 EmA-positive children who were remitted to hospital from primary health care due to suspicion of coeliac disease. None refused to participate nor was immunosuppressive medication. After baseline investigations they were divided into normal villi (Marsh 0-II) and villous atrophy (Marsh III) groups as in studies **I-II.** Thereafter, children with positive EmA but normal villous structure could either continue with a normal gluten-containing diet or start an experimental gluten-free diet at the parents` decision. After one year all the EmA-positive participants were re-investigated. However, as a second gastrointestinal endoscopy after a year on gluten-free treatment is no longer recommended in children (Hill et al. 2005), it was performed only in those who continued gluten consumption. A total of 17 EmA-negative children and adolescents who were investigated because of gastrointestinal symptoms or signs of malabsorption served as non-coeliac controls. All had normal small-bowel mucosal villous structure.

2.2 Changing phenotype of coeliac disease (IV)

The material for Study **IV** comprised three cases who were investigated at the Departments of Paediatrics, Gastroenterology and Alimentary Tract Surgery and Dermatology in Tampere University Hospital between the years 1965-2005. All three had suffered from classical gastrointestinal symptoms of coeliac disease during childhood and at some point were found to have small-bowel mucosal villous atrophy. However, as previously a separate gluten challenge was standard procedure to confirm the diagnosis of coeliac disease (McNeish et al. 1979), in only one of these children was the diagnosis confirmed and a gluten-free diet initiated, whereas in the other two

cases a gluten-containing diet was resumed at puberty. While on challenge, one of these developed villous atrophy and the diagnosis of coeliac disease was established, whereas the other had normal villous structure even after three years on the gluten-containing diet. Thereafter the follow-up of all three cases moved to primary care. After a long asymptomatic period the subjects were remitted to the university hospital with suspicion of dermatitis herpetiformis, and after clinical and serological evaluations a skin biopsy and upper gastrointestinal endoscopy were performed in all three cases.

3. METHODS

3.1 Small-bowel mucosal biopsy samples (I-IV)

The small-bowel mucosal biopsy specimens were taken from the duodenum upon upper gastrointestinal endoscopy. The final processing and evaluation of the samples were completed in the Paediatric Research Centre, University of Tampere. Depending on the study in question, small-bowel mucosal morphology and inflammation (**I-IV**) and TG2-specific autoantibody deposits (**III-IV**) were investigated in the mucosal specimens. All the evaluations were made without prior knowledge of the past medical history, dietary intervention, laboratory values or randomization group of the study subjects.

3.1.1 Mucosal morphology and inflammation (I-IV)

Part of the biopsy specimens were paraffin-embedded and stained with haematoxylin and eosin for morphometric analysis and part were snap-frozen in liquid nitrogen and stored at -70ºC for further use. The small-bowel mucosal morphology was determined from the paraffin specimens under light microscopy from three well-oriented sections. Special attention was paid to the processing and orientation of the biopsy sections and if necessary, the sections were dissected again until they were of sufficient quality. The small-bowel mucosal lesion was scored according to Marsh (1992) as follows: in Marsh 0 there are normal finger-like villi of a normal crypt depth and no excess of

intraepithelial lymphocytes; in Marsh I-II intraepithelial lymphocytosis is seen in normal villous structure without (Marsh I) or with (Marsh II) hyperplasic crypts, and Marsh III comprises villous atrophy with crypt hyperplasia. The mucosal morphology was further more precisely evaluated by measuring Vh/CrD as a mean of at least five well-orientated villous-crypt pairs and a ratio <2.0 was regarded as compatible with untreated coeliac disease (Kuitunen et al. 1982).

Immunohistochemical stainings were carried out on 5-µm-thick frozen sections from three small-bowel mucosal biopsies. CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA, USA), αβ+ IELs with monoclonal βF1 antibody (Endogen, Woburn, MA, USA) and γδ+ IELs with T-cell receptor-γ antibody (Endogen). Positive IELs were counted with a X100 flat field light microscope objective throughout the surface epithelium; at least 30 fields measuring 1.6 mm in epithelial length were counted and IEL density was expressed as cells/mm of epithelium (Arranz et al. 1994). In our laboratory, the correlation coefficient for intraobserver variation for CD3+, αβ+ and γδ+ IELs has been 0.95, 0.85 and 0.98, and for interobserver variation 0.92. 0.82 and 0.98, respectively (Järvinen et al. 2003). Small-bowel mucosal HLA DR expression was measured using monoclonal antibody (Becton Dickinson, San Jose, CA) at a dilution of 1:1000. The expression was considered normal when there was no staining in crypt enterocytes and only slight or moderate expression in the villous epithelium. When any HLA-DR expression was seen on the crypts or was strong in the villous epithelium, the expression was considered enhanced (Arnaud-Battandier et al. 1986)

3.1.2 TG2-specific autoantibody deposits (III-IV)

IgA deposits were investigated in 5-µm-thick frozen sections by direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (DAKO AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline (PBS), pH 7.4. In the mucosa of untreated coeliac patients the autoantibody deposit can be detected on extracellular TG2 below the basement membrane along the villous and crypt epithelium and around mucosal vessels, whereas in non-coeliac subjects IgA is detected only inside plasma and epithelial cells (Korponay-Szabo et al. 2004). The co-localization of IgA deposits with TG2 was

confirmed by double-staining the sections for human IgA and for TG2 using monoclonal mouse antibodies against TG2 (CUB7402, NeoMarkers, Fremont, CA, USA) and anti-mouse immunoglobulin antibodies (DAKO AS, Glostrup, Denmark). In Study III the intensity of the deposits was graded semiquantatively on the basis of the intensity along basement membranes and mucosal vessels in the villous-crypt area as follows: negative, weak (+), moderate (++) and strong (+++) positive. In our laboratory, the intraobserver and interobserver concordances in the detection of IgA deposits have been 98% (Salmi et al. 2006b).

3.2 Serological tests (I-IV)

Serum IgA-class EmA values were determined by an indirect immunofluorescence method using human umbilical cord as substrate, and a patient serum dilution of 1:5 or more was considered positive (Ladinser et al. 1994). If positive, sera were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000 and 1:4000. An enzyme-linked immunosorbent assay (Celikey; Phadia, Freiburg, Germany) was used to investigate the TG2-ab levels (**I-III**). The measurements were carried out according to manufacturer's instructions and values ≥5.0 U were considered positive. In the case of selective IgA deficiency (**III**) both the EmA and TG2-ab values were determined by measuring corresponding antibodies in the IgG class (Sulkanen et al. 1998b, Korponay-Szabo et al. 2003).

3.3 Laboratory parameters (I-II)

Laboratory measurements were made using the standard laboratory methods of Tampere University Hospital. The following parameters were measured in EmA-positive participants: serum albumin (age- and gender-specific reference values of the hospital laboratory 36-48 g/L), serum iron (9-34 μmol/L), red blood cell folate (200-700 nmol/L) and serum vitamin B12 (150-740 pmol/L) (**I**); blood haemoglobin (men 134-167 g/L, women 117-155 g/L), serum ionized calcium (1.20-1.35 mmol/L) (**I-II**); serum parathormone (1.6-6.9 ng/L), (1.20-1.35 mmol/L) and serum vitamin D1,25 (50-215 pmol/L) (**II**).

3.4 Genetic markers (I-III)

The genetic studies to determine the coeliac disease-associated HLA DQ2 and DQ8 genotypes were performed either in the Department of Medical Genetics, University of Helsinki, Finland (**I-III**) or in the Finnish Red Cross Blood Service Laboratory (**I**). The HLA genotype was determined using the DELFIA® Celiac Disease Hybridization Assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) (**I-II**), and the SSP™ DQB1 low-resolution kit (**I-II**) (Olerup SSP AB, Saltsjöbaden, Sweden/ Qiagen Vertriebs GmbH, Vienna, Austria) according to the manufacturer's instructions, or single-nucleotide polymorphisms tagging the coeliac disease-associated HLA haplotypes as described by Monsuur and colleagues (2008) (**III**).

3.5 Clinical symptoms and quality of life (I-III)

In studies **I** and **III**, the researchers evaluated the gastrointestinal symptoms with similar questions at each visit. In study **I** the symptoms were further classified into four categories: no symptoms (0), slight symptoms (1) (occasionally one or more of the following: abdominal pain, flatulence, diarrhoea, belching, tiredness or joint pain), moderate symptoms (2) (symptoms more persistent, disturbing normal life), severe symptoms (3) (severe daily symptoms significantly restricting normal life, or excessive weight loss).

In study **II** the self-rated gastrointestinal symptoms were assessed using the Gastrointestinal Symptoms Rating Scale (GSRS) questionnaire (Svedlund et al. 1988) and health-related quality of life by PGWB questionnaire (Dupuy 1984). The questionnaires are well validated and widely used in coeliac disease research (Hallert et al. 1998, Mustalahti et al. 2002, Usai et al. 2002, Hallert et al. 2002). The GSRS questionnaire comprises 15 items covering five separate gastrointestinal symptoms: diarrhoea, indigestion, constipation, abdominal pain and reflux. Rating is based on a seven-grade Likert scale and higher scores indicate more severe symptoms. In the PGWB questionnaire the 22 separate items comprise six sub-dimensions: anxiety, depression, well-being, self-control, general health and vitality. The scoring is based on a six-grade Likert scale, higher scores indicating better psychological well-being.

3.6 Bone assessment and BMI

The BMD was measured at both the lumbar spine and left femoral neck using DEXA (Sievänen et al. 1996). The density values were expressed as standard deviation scores by comparing individual values either to the mean BMD of sex-matched healthy young adults (T-score) or (subjects under 20 years of age) to that of the age- and sex-matched population (Z-score). As defined by the World Health Organisation (WHO), T-scores above -1.0 represented normal values, scores between -1.0 and -2.4 osteopenia and scores $\leq$-2.5 osteoporosis (WHO 1994). If osteoporosis was detected, bisphosphonate treatment together with supplementary calcium and vitamin D was recommended for ethical reasons. Subjects who started this treatment were excluded from subsequent statistical analyses. Body mass index (BMI) was computed as weight/height^2 (kg/m^2). Values <18.5 were considered underweight, 18.5-24.9 normal, 25.0-29.9 overweight and >30.0 obese (WHO 1998).

3.7 Dietary assessment (I-III)

To ensure strict adherence to the diet, a trained dietitian advised all participants both at baseline and at the end of each trial (**I-III**). In study **III** the children were counselled together with their parents. If a patient was randomized to continue gluten consumption (**I**), the target was to ensure approximately the average daily gluten consumption, i.e 10-15 g of gluten/day (van Overbeek et al. 1997). Adherence to the gluten-free diet was evaluated using combined information obtained from the dietary evaluations, serological markers and histological measurements (**I-III**).

3.8 Statistical analyses (I-III)

Quantative data were expressed as means or medians and ranges (**I-III**), standard deviations (**I**) or 95% confidence intervals (CI) (**II**). When appropriate, chi-square in cross tabulations, two-tailed Student's t-test or Mann-Whitney U test were used to compare differences between groups at baseline (**I-III**) and paired t-test or Wilcoxon signed rank test to compare changes within the groups (**I-II**). A two-tailed P value of less than 0.05 was considered statistically significant. Randomization of the patients

(**I**) was performed using random number tables with permuted blocks (Armitage and Perry 1987).

3.9 Ethical considerations

The study protocols were approved by the Ethical Committee of Tampere University Hospital. All subjects or their parents gave written informed consent.

4. RESULTS

4.1 Patients in the intervention studies (I-III)

The demographic data on the participants are shown in Table 5. In Study **I** there were altogether 70 EmA-positive subjects, of whom 23 had normal small-bowel mucosal villous structure (Marsh I-II), while the remaining 47 had mucosal villous atrophy and crypt hyperplasia (Marsh III). Ten subjects with Marsh I-II were randomized to continue gluten consumption and 13 to start a gluten-free diet. In Study **II,** 27 out of the 73 EmA-positive participants had Marsh I-II and 46 Marsh III. In Study **III** there were 59 EmA-positive children, of whom three (18%) had completely normal mucosal structure (Marsh 0), 14 (82%) had increased IELs (Marsh I) and the remaining 42 Marsh III. Altogether eight children with normal villi continued with a gluten-containing diet and five started dietary treatment; four children moved to other health care districts and were lost to the trial (Figure 1 in original publication **III**). In all three studies EmA-positive subjects were comparable with respect to age, gender and family history of coeliac disease regardless of the mucosal structure. The participants were also comparable to the EmA-negative non-coeliac controls with respect to age and gender (Table 5).

4.2 Small-bowel mucosal morphology and inflammation (I-III)

The results pertaining to the small-bowel mucosal Vh/CrD, IELs and HLA-DR expressions before any dietary intervention are shown in Table 6. As defined in the study protocol, the Vh/CrD was ≥ 2.0 in all EmA-positive participants considered not to have coeliac disease and < 2.0 in all subjects with diagnosed coeliac disease. The baseline levels of the IELs and the mucosal HLA-DR expression were comparable in all EmA-positive participants regardless of the mucosal morphology, except the αβ+ IELs, which were lower in subjects having normal villi in Study **III**. In contrast, these inflammatory markers were significantly higher in the EmA-positive subjects than in the EmA-negative controls (Table 6).

After the baseline investigations ten EmA-positive adults (Study **I**) and eight EmA-positive children (Study **III**) with normal small-bowel mucosal villi remained on a

gluten-containing diet. They were re-endoscopied after one year on trial and the mucosal specimens revealed increasing mucosal deterioration in all adults (Figure 3) and in all but one child (Table 2 in original publication **III**). In contrast, on a gluten-free diet the Vh/CrD increased in all EmA positive subjects regardless of the small-bowel mucosal morphology (Figure 3). Similarly, the IEL counts remained increased after one year on a gluten-containing diet, whereas on a gluten-free diet all except γδ+ IELs decreased significantly (Table 2 in original publication **I**). According to the study protocol the EmA-positive children (Study **III)** were not re-biopsied while on a gluten-free diet.

Table 5. Demographic data, primary reason for coeliac disease suspicion and the human leukocyte antigen (HLA) type of the endomysial antibody (EmA) -positive participants and EmA-negative non-coeliac controls in studies **I-III**

	Study **I**				Study **II**[*]			Study **III**		
	EmA-positive cases				EmA-positive cases			EmA-positive cases		
	Normal villi[†]		Villous atrophy	EmA-negative controls	Normal villi	Villous atrophy	EmA-negative controls	Normal villi	Villous atrophy	EmA-negative controls
	Gluten	GFD								
Number of patients	10	13	47	34	27	46	110	17	42	17
Female, n (%)	6 (60)	9 (75)	41 (87)	21 (62)	18 (67)	40 (87)	89 (81)	10 (59)	25 (60)	10 (59)
Age, median (range), years	53 (16-69)	49 (16-70)	43 (16-71)	53 (22-72)	48 (16-70)	42 (16-70)	49 (24-87)	11 (4-17)	10 (1-15)	11 (5-24)
Primary symptoms n (%)										
Abdominal symptoms	8 (80)	10 (77)	25 (53)	34 (100)	19 (70)	25 (54)	ND	13 (76)	26 (62)	15 (88)
Malabsorption	0	1 (8)	11 (23)	0	3 (11)	11 (24)	ND	3 (18)	5 (12)	1 (6)
Extraintestinal symptoms[‡]	0	1 (8)	5 (10)	0	1 (4)	5 (11)	ND	0	6 (14)	1 (6)
Screening at-risk group[§]	2 (20)	1 (8)	6 (13)	0	4 (15)	5 (11)	ND	1 (6)	5 (12)	0
Duration of symptoms, median (range), years	5 (0-50)	5 (0-40)	4 (0-50)	ND	3 (0-50)	4 (0-57)	ND	1 (0-2)	1 (0-4)	2 (0-4)
Coeliac disease in family, n (%)	5 (50)	5 (45)	16 (34)	0	13 (48)	16 (35)	0	8 (47)	20 (48)	4 (24)
HLA DQ2 or DQ8, n (%)	10 (100)	13 (100)	47 (100)	18 (53)	27 (100)	46 (100)	ND	17 (100)	42 (100)	13 (65)

[*]Participants were partly the same as in Study I
[†]Patients were randomized either to a gluten-containing (Gluten) or a gluten-free (GFD) diet
[‡]Arthritis, dental enamel defects, neurological symptoms, elevated liver enzymes, aphthous stomatitis, gynaecological disorders, osteoporosis, poor growth
[§]Type I diabetes mellitus, autoimmune thyroid disease, Sjögren´s syndrome, family history of coeliac disease.
ND, no data

Table 6. The baseline small-bowel mucosal villous height-crypt depth ratio (Vh/CrD), intraepithelial lymphocytes (IELs, cells/mm) and human leukocyte antigen (HLA) -DR expression of the endomysial antibody (EmA) -positive participants and EmA-negative non-coeliac controls in studies **I-III**

	Study **I**			Study **II**[*]			Study **III**		
	EmA-positive cases			EmA-positive cases			EmA-positive cases		
	Normal villi	Villous atrophy	EmA-negative controls	Normal villi	Villous atrophy	EmA-negative controls	Normal villi	Villous atrophy	EmA-negative controls
Number of patients	23	47	34	27	46	110	17	42	17
Vh/CrD, median	2.6[‡]	0.2[‡]	3.4	2.2	0.2	ND	2.4[†]	0.2[‡]	3.0
Range	2.0-3.6	0.1-1.9	2.8-5.2	2.0-3.6	0.1-1.3	ND	2.1-4.4	0.1-1.4	2.1-3.8
CD3+ IELs, median	62[‡]	63[‡]	32	62	64	ND	50[‡]	80[‡]	28
Range	37-134	34-124	11-62	37-134	34-124	ND	22-64	28-124	14-64
αβ+ IELs, median	37[†]	45[‡]	24	37	45	ND	21	57[‡]	25
Range	16-63	14-104	7-47	16-63	14-104	ND	9-57	20-94	9-68
γδ+ IELs, median	19.1[‡]	19.5[‡]	2.1	19.2	17.9	ND	18.7[‡]	21.9[‡]	2.1
Range	7.0-51.8	5.9-54.6	0.0-24.8	7.0-51.8	5.9-54.6	ND	7.7-41.3	7.6-47.9	0.7-7.7
HLA-DR expression									
Enhanced n (%)	21 (96)[‡]	42 (100)[‡]	18 (53)	25 (96)	44 (100)	ND	16 (94)	42 (100)[‡]	12 (71)
Normal	1 (0)	0 (0)	16 (47)	1 (4)	0 (0)	ND	1 (6)	0 (0)	5 (29)

[*]Participants were partly the same as in Study **I**
[†]P <0.05 compared to EmA-negative controls
[‡]P <0.001 compared to EmA-negative controls
ND, no data

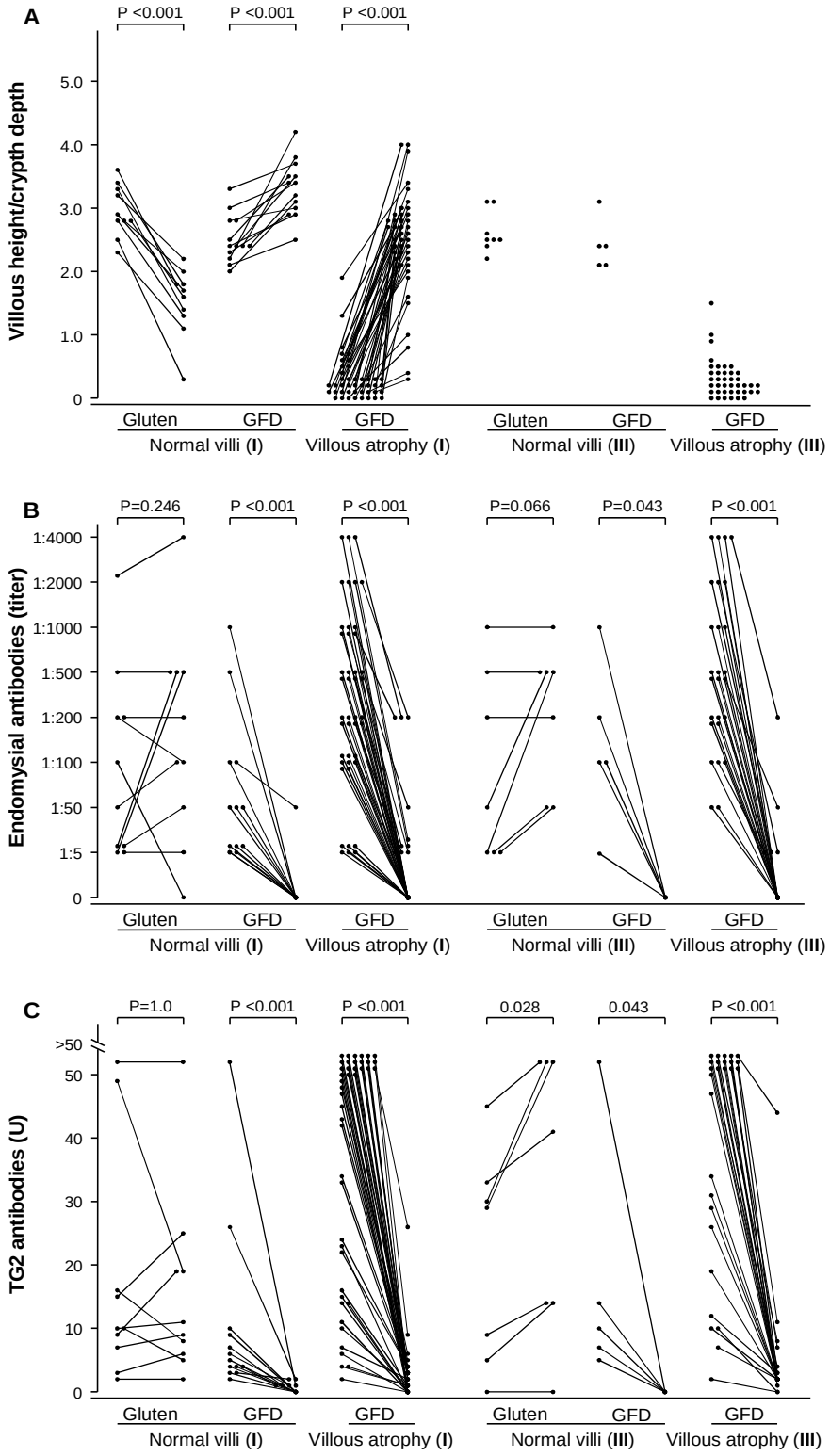

Figure 3. The small-bowel mucosal villous height-crypt depth ratio (Vh/CrD) (**A**) and serum endomysial (EmA) (**B**) and transglutaminase 2 (TG2) (**C**) antibodies at baseline and after one year on a gluten-containing (Gluten) or gluten-free (GFD) diet in the EmA-positive participants in studies **I** and **III.**

4.3 Serology, laboratory parameters and genetics (I-III)

By definition, all participants in the study groups were EmA-positive. The TG2-abs were positive in 14 (61%) out of 23 (Study **I**), 17 (63%) out of 27 (**II**) and 15 (88%) out of 17 (**III**) of those with normal small-bowel mucosal villous structure. The corresponding figures for patients with Marsh III were 44 (94%) out of 47 (**I**), 43 (93%) out of 46 (**II**) and 40 (95%) out of 42 (**III**). Both the EmA titres and the TG2-ab values were significantly lower in participants having normal villi than in those with Marsh III ($P < 0.001$ (**I, II**); $P = 0.002$ (**III**)). One subject in Study **III** was IgA-deficient, but the corresponding antibodies in IgG class were positive. None of the EmA-negative non-coeliac controls had positive TG2-ab values. While on a gluten-containing diet (**I, III**), the EmA titres and TG2-ab values remained or increased in almost all subjects having normal villous structure (Figure 3). In contrast, on a gluten-free diet the antibodies decreased in all subjects regardless of the mucosal morphology. The EmA titres and TG2-ab values either disappeared or decreased likewise in all participants in Study **II.**

In Study **I,** the haemoglobin and iron values were significantly higher in the EmA-positive subjects with Marsh I-II than in those with Marsh III. In participants with normal villi none of the laboratory parameters changed significantly during the dietary interventions, whereas in subjects with Marsh III the haemoglobin, folate and vitamin B12 increased significantly during the gluten-free diet (Table 3 in original publication **I**). In Study **II** the mean haemoglobin level was significantly higher in subjects evincing normal mucosal structure than in those with Marsh III ($P=0.004$). Three patients with Marsh I-II and 12 with Marsh III had parathormone values above reference values. On a gluten-free diet the abnormal parathormone values improved in all of these except for two cases with Marsh III, and the haemoglobin also increased in all subjects (n=9) having anaemia at baseline.

All EmA-positive participants in studies **I-III** had the coeliac disease-associated HLA DQ2 or DQ8 genotype irrespective of the small-bowel mucosal villous morphology, whereas 18 (53%) out of 34 non-coeliac controls in study **I** and 13 (76%) out of 17 in study **III** were DQ2- or DQ8- positive (Table 5).

4.5 Clinical evaluation (I-III) and quality of life (II)

The primary clinical symptoms leading to suspicion of coeliac disease are shown in Table 5. The nature and duration of the symptoms and the number of asymptomatic subjects were comparable in EmA-positive participants irrespective of the degree of small-bowel mucosal damage. When the EmA-positive participants with normal villous structure continued gluten consumption the symptoms remained basically unaltered, whereas on a gluten-free diet they either disappeared or were alleviated in almost all subjects regardless of the mucosal structure (Figure 3 in original publication **I** and Table 2 in original publication **III**).

There were no differences in the GSRS total or sub-dimension scores between the Marsh I-II and Marsh III groups, except for a higher frequency of diarrhoea in the latter (Study **II**). However, the participants in both EmA-positive groups had significantly more diarrhoea, indigestion, abdominal pain and reflux than the non-coeliac controls (Table 2 in original publication **II)**. On a gluten-free diet the indigestion score in the Marsh I-II and the total and all sub-dimension scores in the Marsh III group decreased significantly (Figure 2 in original publication **II**). The differences in the PGWB total or sub-dimension scores were not significant between the EmA-positive study groups and the non-coeliac controls at baseline. While on a gluten-free diet the depression score in the Marsh I-II group and total, self-control and general health scores in the Marsh III group improved significantly (Figure 3 in original publication **II**).

4.6 Bone assessment and BMI (II)

At baseline, 11 (58%) out of 19 subject with EmA but normal villi had either osteopenia (47%) or osteoporosis (11%). The mean Z-score was -0.1 (95% CI -0.5 to 0.4) for the lumbar spine and 0.1 (-0.4 to 1.0) for the femoral neck. Of the Marsh III subjects 20 (51%) out of 39 had osteopenia and eight (21%) osteoporosis, and the mean Z-scores were -0.6 (-1.0 to -0.1) for lumbar spine and -0.3 (-0.6 to -0.1) for femoral neck. After the year on a gluten-free diet the BMD was re-measured in the 10 subjects with normal villi, and although BMD increased in most of them, the changes were not statistically significant (Figure 4 in original publication **II**). In the Marsh III group BMD increased significantly in both the lumbar spine and femoral neck.

The BMI was within normal range in 11 (41%) out of 27 subjects with Marsh I-II, 12 cases (44%) were overweight and four (15%) obese. In the Marsh III group one patient (2%) was underweight, 23 (55%) were normal-weight, 10 (24%) overweight and eight (19%) obese. Differences between the groups or changes within the groups while on treatment were not statistically significant.

4.7 Changing phenotype of coeliac disease (IV)

All three patients in this study had an itching bullous rash suggestive of dermatitis herpetiformis, whereas none had significant gastrointestinal symptoms (Figure 4). The asymptomatic period from the last endoscopic investigations ranged from 13 to 18 years, and during this time the subject with negative gluten challenge (Case 2) had remained on a gluten-containing diet and in the other two cases dietary treatment had been incomplete. Skin biopsy of all three cases showed granular IgA deposits in the dermal papillae of the perilesional skin, confirming the diagnosis of dermatitis herpetiformis. Although at some point they all had gastrointestinal presentation and small-bowel mucosal villous atrophy, at the time of the dermatitis herpetiformis diagnosis there was no intestinal symptoms and only partial villous atrophy in the mucosa. In addition, all cases had either positive ARA or EmA in the serum. The small-bowel mucosal TG2-specific IgA deposits were also measured from frozen sections and all three patients evinced clear coeliac-type deposition (Figure 2 in original publication **IV**). Subsequently all three patients were again placed on a strict gluten-free diet, with a good clinical response and negative seroconversion of the coeliac antibodies.

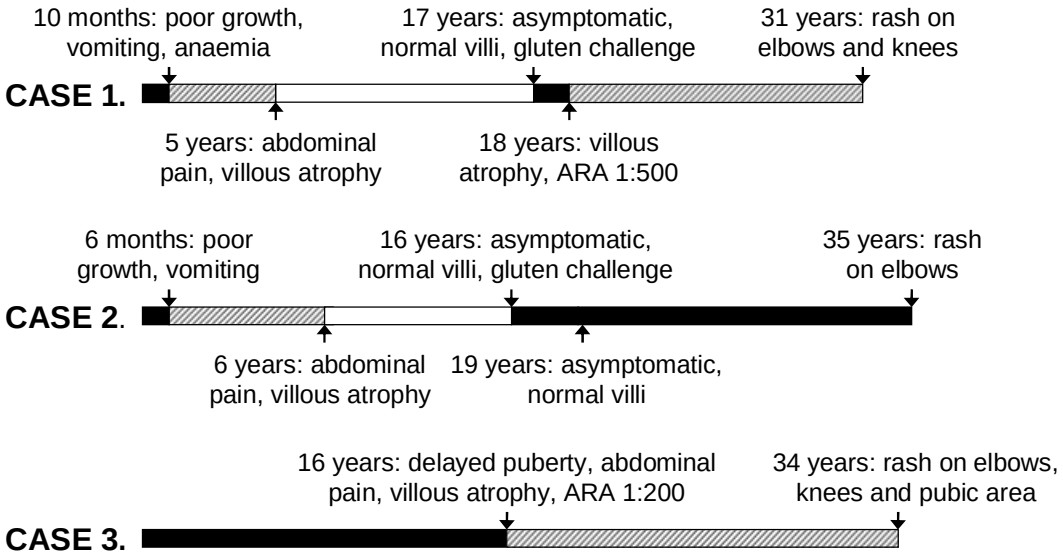

Figure 3. The medical history of the three patients in Study **IV**. Black bars = gluten-containing diet; white bars = gluten-free diet; dashed bars = partial gluten-free diet. ARA, antireticulin antibodies

4.8 Dietary choices after the trial (I-III)

After the trials were completed, all the EmA-positive participants in studies **I-III** were considered to suffer from a gluten-dependent disorder regardless of the small-bowel mucosal morphology. Thus, a gluten-free diet was offered, and either at their own (**I-II**) or by their parents (**III**) decision all continued on treatment.

5. DISCUSSION

5.1 Problems with the current histological criteria

The diagnostic criteria for coeliac disease have developed together with an increasing understanding of the natural history of the disorder. As the decision to start out on life-long treatment has to be based on firm evidence, the first criteria required altogether three separate intestinal biopsies to ensure the gluten-dependency of the patients (Meeuvisse 1970). As a result, the diagnostic protocol was particularly burdensome and long drawn-out. In addition, it was eventually discovered that mucosal recovery while on treatment might take several years (Grefte et al. 1988), and on the other hand, the histological relapse while on a gluten challenge could emerge much later than the two years required for the diagnosis (Kuitunen et al. 1986). These observations led in 1990 to the establishment of new and simplified diagnostic criteria (Walker-Smith et al. 1990). Nevertheless, demonstration of small-bowel mucosal villous atrophy was still required and the few recent updates have not markedly changed this situation (United European Gastroenterology 2001, Hill et al. 2005).

Unfortunately, substantial problems attend this histology-based definition of coeliac disease. The mucosal lesion can be patchy along the whole length of the small intestine and might be missed even if several biopsies are taken (Scott and Losowsky 1976). Furthermore, imprecise slicing or wrong orientation of the biopsy samples may yield erroneous results and, even if properly processed, the final analysis of the specimens is always somewhat subjective (Collin et al. 2005a). Importantly, none of the currently known histopathologic markers is pathognomic for coeliac disease as such (Kuitunen et al. 1975, Spencer et al. 1991, Iltanen et al. 1999b, Kakar et al. 2003, Lähdeaho et al. 2005). Finally, the current diagnostic criteria focus mainly on the classical gastrointestinal disorder and ignore extraintestinal forms of coeliac disease such as dermatitis herpetiformis.

Apart from histological assessment, another question has been the role of the coeliac antibodies in the diagnosis. The first serological markers of coeliac disease were discovered as far back as the early 1970s (Seah et al. 1971, Carswell and Ferguson 1972), but their specificity was considered insufficient for a definitive diagnosis (Unsworth et al. 1983), although later the IgA-class ARA showed excellent results in an experienced laboratory (Mäki et al. 1984b). Furthermore, although the

highly specific EmA were already established by Chorezelski and associates in 1983, they were widely accepted only after human tissue could be used as substrate; that is, after the current diagnostic criteria were launched (Ladinser et al. 1994). The establishment of the TG2-ab tests a few years later (Dieterich et al. 1998, Sulkanen et al. 1998a) practically revolutionized the diagnostic approach, but thus far the role of the autoantibodies in the diagnosis of coeliac disease has remained mainly supportive (United European Gastroenterology 2001, Hill et al. 2005).

5.2 Coeliac disease without villous atrophy

The previous section emphasized the technical problems and unsolved questions related to the diagnosis of coeliac disease, but the situation became even more challenging when it was discovered that the characteristic small-bowel mucosal damage develops gradually (Mäki et al. 1990, Mäki et al. 1991a, Marsh 1992). This observation and the fact that patients may be symptomatic while still having villi indicate that the current diagnostic criteria should be revisited (Kaukinen et al. 2001). Although mild mucosal changes are an unspecific finding as such (Kuitunen et al. 1982, Lähdeaho et al. 2005), it has been suggested that the more specific EmA and TG2-abs could be used for early diagnosis when the villous structure is still normal (Kaukinen et al. 2001, Dickey et al. 2005, Paparo et al. 2005, Koskinen et al. 2008). However, firm evidence for new criteria has been lacking and the present series (**I-III**) were the first controlled, prospective clinical studies to evaluate the natural history and effect of a dietary intervention in subjects having positive EmA but normal small-bowel mucosal structure.

5.2.1 Histology, serology and genetic markers

To obtain objective results, the small-bowel mucosal morphology was evaluated using the Vh/CrD as described by Kuitunen and associates (1982). Interestingly, although the Vh/CrD was by definition ≥2.0 in all EmA-positive adults and children evincing normal villi, the ratios increased even further while these subjects were on a gluten-free diet, and thus the “normal” small-intestinal mucosa improved even further. In contrast, on a gluten-containing diet the Vh/CrD decreased in almost all EmA-positive

subjects; in fact, many of them developed villous atrophy (Figure 3A). These results demonstrated that both the development of the mucosal damage and its recovery are gradual and gluten-dependent processes. Based on these findings, there is no clear morphological cut-off line which would separate EmA-positive "non-coeliac" patients from those with gluten-sensitive disease. Since villous damage may also appear in conditions other than coeliac disease (Kuitunen et al. 1982, Green and Cellier 2007), it seems that these structural changes have limited value in the diagnosis at least of borderline cases.

The small-bowel mucosal inflammation was measured using several different histological markers of coeliac disease. In adults the CD3+ and αβ+ IELs and the mucosal HLA-DR expression were comparable between the EmA-positive groups, but differed markedly from the EmA-negative controls. Furthermore, the IELs showed clear gluten-dependent responses during the dietary interventions. Although in children the CD3+ and αβ+ IELs were higher in those with villous atrophy than in those having normal villi, the cell densities were still increased in both groups when compared with the EmA-negative controls. The results demonstrated that, similarly to the morphological changes, the development of the mucosal inflammation is a gradual and gluten-dependent process. Nevertheless, consistent with previous studies there was substantial overlapping between EmA-positive and EmA-negative participants, showing again that these inflammatory markers are too unspecific for the diagnosis as such (Kuitunen et al. 1982, Järvinen et al. 2003, Kakar et al. 2003, Salmi et al. 2010).

In contrast, the increased density of the mucosal γδ+ IELs is a much more specific finding (Spencer et al. 1991, Järvinen et al. 2003) and the high density of these cells in EmA-positive subjects with normal villi gives further evidence for the presence of a coeliac-type disorder. However, even the γδ+ IELs were elevated in some EmA-negative subjects, and since they may appear without coeliac-type HLA (Iltanen et al. 1999b), it is evident that they are not pathognomic for coeliac disease. In addition, as also noted in previous studies (Järvinen et al. 2003 and 2004), the γδ+ IELs showed a rather slow and inconsistent response to the dietary treatment. Given that special methods and a frozen sample are required for their determination, it would seem that the γδ+ IELs seldom provide any additional benefit in EmA-positive cases.

Irrespective of the mucosal morphology, the serum EmA titres showed indisputable gluten-dependent responses to the dietary interventions, again emphasizing the similarity of the antibody-positive participants. In addition, the TG2–abs showed a

strong correlation to the EmA levels and evinced corresponding responses to the gluten-free diet. This was quite as expected, since both antibodies are targeted against TG2 (Korponay-Szabo et al. 2003) and have shown similar specificity figures in previous studies (Table 2). Besides the antibodies in serum, the coeliac-type intestinal IgA deposition was present in all the EmA-positive children but in none of the seronegative controls (Study **III)**. Since the deposits have been shown to be highly specific for forthcoming coeliac disease (Korponay-Szabo et al. 2003, Kaukinen et al. 2005, Salmi et al. 2006b, Koskinen et al. 2008), their presence in the EmA-positive children was a very strong indicator of a coeliac-type disorder.

Despite similar responses to the dietary interventions, the EmA titres and TG2-ab values were significantly lower in subjects with normal villi than in those having villous atrophy. This is consistent with recent findings by Donaldson and associates (2007), where the antibody levels were also lower in children with normal villi than in those having Marsh III. Furthermore, Abrams and colleagues (2004) have shown that subjects having partial villous atrophy are more often seronegative than those with completely flat mucosa. The reason for these differences is unclear, but it is of note that both here and in a study by Koskinen and colleagues (2008) the intensity of the mucosal IgA deposits correlated with the degree of enteropathy. These results suggest that the reason for the lower serum autoantibody levels in patients with normal villi is reduced intestinal production. Finally, regardless of the small-bowel mucosal morphology, all the EmA-positive subjects here had either the HLA DQ2 or the DQ8 genotype. Since these haplotypes are practically a requirement for coeliac disease, their presence gives further evidence that all these EmA-positive participants belonged to the genetically gluten-sensitive population (Sollid et al. 1989, Mäki et al. 2003, Karell et al. 2003).

5.2.2 Clinical evaluation, quality of life and dietary compliance

The clinical symptoms were evaluated both by the researchers and using structured questionnaires and, except for diarrhoea, they were comparable irrespective of the small-bowel mucosal morphology. Similarly, the laboratory values were mostly at the same level regardless of the villous structure. This rather small correlation between the clinical presentation and the degree of enteropathy was somewhat unexpected, as it

would seem logical that patients with villous atrophy would have more symptoms. However, there are also previous studies showing no obvious correlation between the clinical symptoms and the degree of mucosal damage (Brar et al. 2007, Murray et al. 2008, Table 3). Instead, it has been suggested that the extent of the intestinal involvement would determine the clinical severity of coeliac disease (Marsh and Crowe 1995), but even this hypothesis has recently been questioned (Murray et al. 2008). Nevertheless, there were still differences between the EmA-positive groups in diarrhoea and some laboratory parameters. This is in line with findings by a group under Donaldson (2007), who noted that EmA-positive children with normal villi had less diarrhoea than those with Marsh III. It could thus be speculated that only the malabsorptive symptoms are directly related to the villous damage, while most of the clinical symptoms are caused by other factors. In any case, the results confirmed previous findings that EmA-positive subjects may suffer from clinical symptoms and benefit from dietary treatment while still having normal mucosal morphology (Kaukinen et al. 2001, Paparo et al. 2005, Dickey et al. 2005, Salmi et al. 2006).

In the past few decades the self-assessed quality of life has gained increasing attention in medical research, since it provides information as to how a particular disease affects the daily lives of patients (Yacavone et al. 2001). This is especially important in coeliac disease, where the treatment involves life-long and demanding dietary restriction. It was somewhat surprising that no significant differences were observed between the EmA-positive participants having either normal villi or villous atrophy and the non-coeliac controls. Nonetheless, there was a trend towards poorer PGWB scores in the EmA-positive groups and it is possible that their number was too small for the results to reach statistical significance. Alternatively, general questionnaires such as PGWB might not be sensitive enough to reflect minor abnormalities and more disease-specific instruments might have been needed (van Doorn et al. 2008). The assumption that these subjects still had reduced quality of life is supported by the fact that some of the PGWB subscores improved significantly during the dietary intervention. Particularly the reduction in depression in subjects with normal villi was important, since depression has a been common finding in untreated coeliac disease and may also reduce dietary compliance (Ciacci et al. 1998, Ludviggson et al. 2007, Nachman et al. 2009). The fact that none of the PGWB subscores decreased in the EmA-positive groups further supports the overall benefits of a gluten-free diet. Finally, it must be emphasized that according to the current

criteria these EmA-positive and symptomatic subjects would have to undergo regular and burdensome endoscopic studies and long-term follow up until villous atrophy develops.

In contrast to some other studies (Högberg et al. 2003, Whitaker et al. 2009), the dietary compliance of the EmA positive participants was excellent throughout the present series. Although it is possible that those who participated were more willing to start the treatment than those who declined, good dietary compliance has been a frequent finding also in other Finnish studies of coeliac disease (Viljamaa et al. 2006). These results may be partly explained by the high prevalence of diagnosed cases and the generally good knowledge about coeliac disease in Finland (Virta et al. 2009). Furthermore, the availability of gluten-free products from groceries is good and biopsy-proven patients obtain monthly financial support from the Social Insurance Institution. In any case, the present study showed that good dietary compliance can be achieved irrespective of the small-bowel mucosal structure.

5.2.3 Bone assessment and BMI

As expected in the light of previous studies (Caraceni et al. 1988, Valdimarsson et al. 1994, Corazza et al. 1995b), the baseline BMD was significantly decreased in subjects with small-bowel mucosal villous atrophy. Nevertheless, decreased BMD was also noted in many EmA-positive subjects with normal villi. This is in accord with other recent studies suggesting that coeliac disease can predispose to osteoporosis even prior to the development of villous atrophy (Kaukinen et al. 2001, Tursi et al. 2003, Dickey et al. 2006, Esteve et al. 2006). The reason for this reduction in BMD is obscure, but a similar tendency to osteoporosis has also been observed in patients suffering from inflammatory bowel disease, indicating that chronic intestinal inflammation might have an adverse effect on bone mineral accrual (Taranta et al. 2004, Tilg et al. 2008). Although the clinical relevance of the decreased BMD is unclear, the fact that subjects with villous atrophy are at significantly increased risk of bone fractures (Vasquez et al. 2000, West et al. 2003a) suggests that early-initiated treatment might lower this risk in such patients. In addition, delayed treatment of coeliac disease in childhood predisposes to permanent disturbances in bone maturation and growth, further

emphasizing the importance of early diagnosis (Barr et al. 1966, Mora et al. 1999, Tau et al. 2006).

Contrary to the classical picture of "withered" coeliac disease patients, the EmA-positive subjects evinced a proneness to overweight regardless of the mucosal morphology. In fact, a tendency towards obesity similar to that in the general population has also been observed in other recent studies of coeliac disease (West et al. 2004b, Viljamaa et al. 2005, Dickey and Kearney 2006). The observation may reflect the changing pattern of coeliac disease, as nowadays patients often have milder clinical symptoms at diagnosis than previously (Mäki et al. 1988a, Green and Cellier 2007). Interestingly, there was no difference in the EmA-positive groups in either the mean BMI between the groups or within the groups during the gluten-free diet. These results are somewhat contradictory to those obtained by Dickey and Kearney (2006), where subjects with severe villous atrophy had significantly lower BMI than those with milder mucosal damage, and even overweight patients gained more weight while on treatment. Although these disparities may be partly explained by the smaller number of cases in the present series, the results still suggest that a gluten-free diet is not necessarily detrimental to the weight control of coeliac patients.

5.3 Genetic gluten intolerance

While the prospective studies here (**I-III**) demonstrated that EmA positive subjects have a similar gluten-dependent disorder irrespective of the villous morphology, Study **IV** showed that the clinical and histological phenotype of coeliac disease can change over time. At some point all three patients in this group had gastrointestinal symptoms and overt villous atrophy, but at the time of their skin relapse they had only skin symptoms and partial villous atrophy in the mucosa. In addition, in one case the villous structure was normal after three years on gluten challenge, showing that there can be long periods of histological latency. It is well known that the mucosal damage in dermatitis herpetiformis may be variable (Marks et al. 1966, Savilahti et al. 1992, Reunala 2001), but hitherto the natural history of the histological changes has remained unclear. However, it has been shown that identical twins may have disconcordance in the clinical and histological presentation, with intestinal disease in one and dermatitis herpetiformis in the other sibling (Hervonen et al. 2000).

Interestingly, all three cases also had positive ARA or EmA in the serum and TG2-targeted IgA deposition in the small-bowel mucosa, again showing that the autoantibodies are the integrative feature in the distinct forms of coeliac disease. Furthermore, the presence of the autoantibodies is a very early phenomenon in the disease process (Korponay-Szabo et al. 2004, Simell et al. 2007) and there is increasing evidence that they also have an important role in the pathogenesis (Halttunen and Mäki 1999, Esposito et al. 2002, Barone et al. 2007, Myrsky et al. 2008). It has since been speculated that after being produced in the intestine the antibodies may enter the circulation and participate in the extraintestinal manifestations of coeliac disease (Korponay-Szabo et al. 2004, Lindfors et al. 2009). For example, subjects with autoantibodies against TG3 could develop dermatitis herpetiformis (Sardy et al. 2002) and subjects with those against TG6 gluten ataxia (Hadjivassiliou et al. 2008b). The same patient could still have antibodies against TG2 and simultaneous intestinal disease or even fluctuating phenotype, as in Study **IV**. Such a conception is supported by the fact that subjects with dermatitis herpetiformis diagnosed in adulthood may have coeliac-type permanent tooth enamel defects, suggesting that some form of gluten sensitivity has been present in early childhood (Aine 1996).

These results indicate that both the intestinal and extraintestinal forms of coeliac disease share the same genetic background, dependency on dietary gluten and initial intestinal pathogenesis with or without a manifest mucosal lesion. Thus, instead of coeliac disease, the term *genetic gluten intolerance* might be more descriptive for the wide spectrum of these disorders. Interestingly, already in the 1990s a so-called "cooking-pot" model of coeliac disease was presented by Mäki (1992). In this model the intestine is the primary "pot" for the pathogenetic events, and the clinical manifestations are the end-result of the genetic predisposition, gluten exposure and other environmental factors (Kaukinen et al. 2007b). In 1997 Mäki and Collin also suggested using the term *coeliac trait* for this broadened view of gluten-dependent disorders. The results of the present series and the knowledge of the extraintestinal forms of coeliac disease accruing in recent years provide further evidence for this model (Figure 5).

Besides changing phenotype, the clinical relapses in Study **IV** occurred after a particularly long asymptomatic period. This is consistent with other findings indicating that the histological and clinical relapse may take even decades despite continuous

gluten consumption (Kuitunen et al. 1986, Högberg et al. 1993, Matysiak-Budnik 2007). This is important, since the increasing serological screening often reveals cases with positive antibodies but no other signs of coeliac disease and it is still debated whether some cases could remain in permanent latency (Simell et al. 2007, Matysiak-Budnik et al. 2007). Accordingly, there was also one child in Study **III** in whom the mucosal structure remained normal despite a gluten-containing diet. Then again, her coeliac antibodies persisted and it is possible that she will develop either villous atrophy or more severe clinical symptoms in the future. However, for the moment the natural history of the EmA-positive but asymptomatic cases with normal villi is unclear and only careful follow-up can be recommended (Simell et al. 2010).

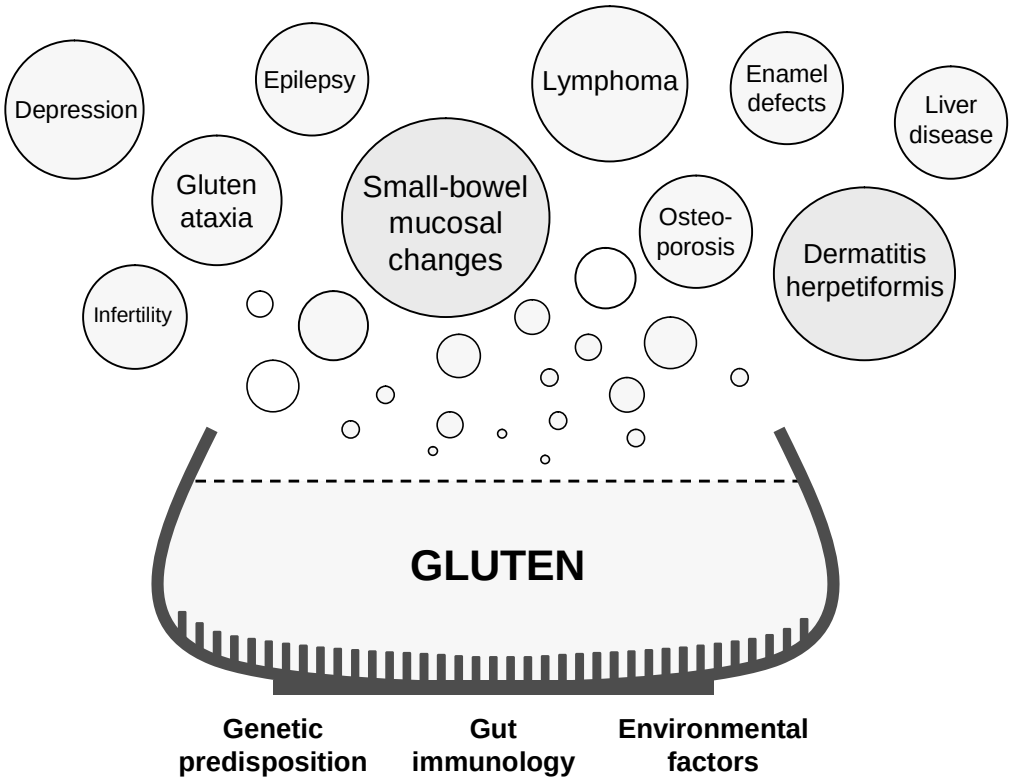

Figure 5. The cooking pot of coeliac disease. The development of coeliac disease requires a complex interaction between genes, environmental factors and the immunological system of the gut. Although the small intestine is the principal site for the pathogenetic mechanisms, the mucosal lesion represents only one entity among the various clinical manifestations of genetic gluten intolerance. Adapted from Mäki 1995 and Kaukinen et al. (2007b).

5.4 Limitations of the study and future challenges

Certain important issues should be considered when interpreting the results of this study. First, there was a small number of subjects who either refused to participate or were excluded from the studies, and in theory they might have affected the results obtained. In addition, for ethical reasons the paediatric study was conducted as a non-randomized follow-up trial. However, no significant difference in either clinical symptoms, antibody levels or histological markers was observed between the children who remained on a normal diet and those who started the dietary intervention (Table 2 in original publication **III**). Any risk that gender distribution might have affected to the results is unlikely, since the groups were comparable in this respect and the apparent over-representation of females is characteristic of coeliac disease (Green et al. 2001).

The use of highly specific EmA as an inclusion criterion was fairly straightforward, but there are certain problems related to the use of the immunofluorescence method. First, it is laborious and time-consuming and thus unpractical for large-scale screening studies. Secondly, the interpretation of the specimens is inevitably subjective, which may in borderline cases comprise a risk of a wrong negative or positive result. The positive EmA staining can also be masked by the presence of other serum immunoglobulins such as anti-smooth muscle antibodies (Sulkanen et al. 1998b). Finally, the good accuracy of EmA is shown primarily in subjects with a high pre-test probability of coeliac disease, and those having mild symptoms or lesser degrees of mucosal damage may yield poorer results (Rostami et al. 1999, Abrams et al. 2004). For the aforementioned reasons it is essential that only well-validated laboratories and qualified personnel are used if EmA positivity is to constitute a diagnostic criterion for coeliac disease.

In contrast to EmA, TG2-abs can be measured using a practical and objective ELISA method, and it would be tempting to generalize the results of the present study to include subjects with positive TG2-abs. Indeed, most of the EmA-positive participants had positive TG2-abs and their gluten-dependency was shown by measuring both antibodies. On the other hand, as the TG2-abs are often used as a first-line screening method, it has been essential to obtain maximal sensitivity at the expense of optimal specificity. Furthermore, the accuracy of the commercial TG2-ab assays seems to be somewhat variable and method-dependent (Villalta et al. 2005,

Hopper et al. 2007). Consequently, the results of this study should not be applied to subjects having only positive TG2-abs.

Most of the EmA-positive participants in the present study had clinical symptoms and only few were screen-detected asymptomatic subjects. As a consequence, the results can not be generalized to subjects having positive EmA and normal villi but no symptoms. This is problematic, since serological screening has revealed that asymptomatic subjects may actually represent a substantial proportion among coeliac patients (Mäki et al. 2003, Fasano et al. 2003, Korponay-Szabo et al. 2007). In fact, although a gluten-free diet is recommended to asymptomatic patients with villous atrophy, no randomized studies have ever been undertaken and actual evidence for their treatment is scant (Hill et al. 2005). Nevertheless, as there is some evidence that they may benefit from the dietary treatment, it has been speculated whether even population-based screening for coeliac disease would be justified (Mustalahti et al. 1999, Viljamaa et al. 2004, Fasano 2009). Evidently, more studies are needed, but at present it seems reasonable that at least patients with any symptoms or signs of coeliac-disease associated complications should be treated regardless of the clinical presentation (Collin 2005b).

Finally, the results lead to the inevitable question whether a small-bowel biopsy is necessary for all subjects with positive EmA and clinical symptoms. In fact, the need for histological confirmation of the diagnosis was questioned soon after the excellent specificity of EmA for coeliac disease was observed (Valdimarsson et al. 1996). Our results show that EmA-positive patients have a similar gluten-dependent disorder and should be treated regardless of the degree of the mucosal damage, and it would seem logical that at least some of these subjects could be diagnosed without histological confirmation. Nevertheless, the present study was not designed to evaluate the role of endoscopic studies in the diagnostics. In addition, there were only a few cases with completely normal mucosa and the possible benefits of the dietary treatment in these subjects calls for further confirmation. Thus, in the future more randomized studies are needed to assess the role of intestinal biopsy in the diagnosis of coeliac disease.

6. SUMMARY AND CONCLUSIONS

The present study demonstrated that the current diagnostic criteria for coeliac disease are inadequate to cover the wide clinical spectrum of the gluten-dependent disorders. Firstly, the prospective studies (**I-III**) showed that EmA positive subjects may suffer from the clinical symptoms and even complications of untreated coeliac disease irrespective of the small-bowel mucosal morphology. Furthermore, the gluten dependency of these subjects was demonstrated using several histological, clinical and serological markers. The results confirmed the hypothesis that villous atrophy represents only the end-point in the gradual development of the histological changes in coeliac disease. It was also observed that none of the currently known histological markers, for example CD3+ and γδ+ IELs or mucosal HLA-DR expression, is specific for an early developing disease. Based on our results, at present determination of serum EmA and intestinal TG2-targeted IgA deposits is the most accurate diagnostic method for coeliac disease.

The result of Study **II** showed that early intervention with a gluten-free diet was detrimental for neither the quality of life nor the weight control of the EmA-positive subjects with normal villi. Furthermore, there was a trend towards increasing BMD values in these participants, suggesting that early treatment might at least prevent the development of more severe osteoporosis. These findings are important, since life-long treatment with gluten-free diet can be restrictive and difficult to maintain, and it was essential to show that early treatment is not harmful for the daily life of these EmA-positive participants. The conclusion that the dietary treatment was beneficial was further supported by the fact that irrespective of the degree of villous damage, all EmA-positive participants continued on a gluten-free diet when the trials were completed.

Finally, the results of Study **IV** demonstrated that both the histological and the clinical presentation of coeliac disease can fluctuate over time, and that even the complete phenotype of the disorder may change from intestinal to extraintestinal. The fact that all three patients had positive serum autoantibodies and TG2-targeted mucosal IgA deposits constitutes further evidence that the two forms of coeliac disease are merely different phenotypes of the same gluten-dependent disorder.

In conclusion, the present study confirmed that small-bowel mucosal villous atrophy is not the optimal golden standard for coeliac disease and that there are a

substantial number of patients who have an indisputable gluten-sensitive disorder while still evincing normal mucosal morphology. Furthermore, the intestinal and extraintestinal forms of coeliac disease are only different variations of the same gluten-dependent disorder. Based on the findings here, in symptomatic subjects having only inflammatory changes in the small-bowel mucosa EmA positivity represents a sufficient and highly specific marker of a genetic gluten-sensitive disorder. In the future, more prospective studies are needed to elucidate the natural history of EmA-positive subjects who manifest no clinical symptoms and those with completely normal intestinal mucosa. Also, randomized studies are required to assess whether the coeliac autoantibodies can completely replace the conventional histology in the diagnosis of the disorder.

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