SINI LOHI

Prevalence and Prognosis of Coeliac Disease

A special focus on undetected condition

ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the Auditorium of Finn-Medi 1,
Biokatu 6, Tampere, on January 22nd, 2010, at 12 o’clock.

UNIVERSITY OF TAMPERE
“When spiders’ webs unite, they can tie up a lion.”

Ethiopian proverb

To my loved ones
ABSTRACT

Thirty years ago coeliac disease was considered a rare disease affecting approximately 0.01% of Western populations. Since then, with greater awareness of disease manifestations and the development of serological tests for coeliac autoantibodies, the incidence of the diagnosed disorder has increased many-fold. Independent of this, population screening studies since the 1990s have uncovered a number of undetected cases and prevalence figures of roughly 0.5-1% have been reported on all continents of the world. Thus, the aim of the current study was to establish whether a true rise in coeliac disease prevalence is in fact under way. The aim was also to establish whether unrecognized cases involve an increased risk of malignancies and mortality.

The Mini-Finland (1978-80) and Health 2000 (2000-01) population-based surveys enabled comparison of prevalence figures two decades apart. In parallel with previous reports, the prevalence of diagnosed adult coeliac disease increased substantially from 0.03% to 0.52% during the time-span examined, while independent of better diagnostics, the prevalence of previously unrecognized screen-detected cases in the current study also increased over time, from 1.03% to 1.47%. It was thus shown for the first time that the total prevalence of coeliac disease, including both clinically diagnosed and screen-detected cases, has increased in the course of time. The figure nearly doubled, from 1.05% to 1.99%, in two decades, which is in line with the increasing figures in other autoimmune diseases such as type 1 diabetes, and allergic conditions.

The substantial number of unrecognized coeliac cases has also prompted debate as to whether the disorder should be diagnosed as early as possible, even by population screening programmes. However, the necessity of such programmes has been questioned, since the natural history of the condition is largely unknown. Prognostic studies so far have concentrated on diagnosed and thus treated cases. The second aim in the present study was therefore to establish whether unrecognized cases, similarly to those diagnosed, involve an increased risk of malignancies and
mortality. In the present study, earlier undetected and thus untreated coeliac autoantibody-positive subjects in the Mini-Finland survey (1978-80) were compared to negative subjects in respect of malignancies and mortality in long-term surveillance. No increased risk of overall malignancy or mortality was detected. Nonetheless, the risk of non-Hodgkin lymphoma, stroke and respiratory system diseases was emphasized, a finding which remains to be confirmed in future studies.

All in all, the total prevalence of coeliac disease nearly doubled during the two decades studied and further, the prognosis of undetected coeliac disease is good as regards the overall risk of malignancies and mortality.
Vielä kolmekymmentä vuotta sitten keliakiaa pidettiin varsin harvinaisena sairautena ja sen esiintyvyydeksi eli prevalenssksi länsimaissa arvioitiin 0.01 %. Sittemmin keliakian ilmaantuvuus on moninkertaistunut sekä sairauden oirekirjon paremman tuntemisen että keliakiavasta-aineiden käyttöönoton seurauksena. Tästä huolimatta 1990-luvulta lähtien tehdyt seulontatutkimukset ovat paljastaneet huomattavan määrän tunnistamattomia tapauksia ja osoittaneet karkeasti 0.5-1.0 % esiintyvyyden kaikissa maanosissa. Tämän tutkimuksen tavoitteena oli selvittää, onko keliakian esiintyvyydessä tapahtunut todellista lisääntymistä. Tavoitteena oli myös tutkia, onko tunnistamatonta keliakia sairastavalla lisääntynyt syöpä- ja kuolleisuusriski.

Prevalenssien vertailu onnistui Mini-Suomi (1978-80) ja Terveys 2000 (2000-01) -tutkimusaineistojen avulla. Diagnosoitujen tapausten määrä nousi 0.03 %:sta 0.52 %:iin tutkittuna aikana, mikä on sopusoinnussa aikaisempien tutkimusten kanssa. Parantuneesta diagnostiikasta huomattava myös seulomalla löydettyjen tietämättään keliakiaa sairastavien osuus kasvoi 1.03 %:sta 1.47 %:iin tässä tutkimuksessa. Tätä tutkimuksessani voitiin ensimmäistä kertaa osoittaa sairauden kokonaisesiintyvyyden nousseen ajan kuluessa. Prevalenssi sisälsi sekä aikaisemmin diagnosoidut että seulonnalla löytyneet tapaukset. Tämä kokonaisprevalenssi nimitään lähes kaksinkertaistui 1.05 %:sta 1.99 %:iin kahden vuosikymmenen kuluessa. Vastaavanlaisesta esiintyvyyden kasvusta on raportoitu myös muiden autoimunuaisairauksien, kuten tyyppi 1 diabeteksen ja allergisten sairauksien, yhteydessä, mikä on sopusoinnussa tässä tutkimuksessa havaitun keliakian lisääntymisen kanssa.

Tunnistamattomien keliaakikoiden suuri määrä on herättänyt keskustelua myös siitä, pitäisikö nämä tapaukset todeta mahdollisimman varhain, jopa väestöseulonnan avulla. Seulonnat on kuitenkin kyseenalaistettu, koska tunnistamattoman keliakian luonnollinen kulku on ollut suurelta osin epäselvä. Ennustetutkimukset ovat toistaiseksi keskittyneet diagnosoituihin ja siten

Yhteenvetona voidaan todeta, että keliakian kokonaisprevalenssi on lähes kaksinkertaistunut kahden vuosikymmenen aikana. Tunnistamattomaan keliakiaan ei näytä liittyvän lisääntynytä yleistä syöpää- tai kuolleisuusriskiä.
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LIST OF ORIGINAL PUBLICATIONS

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


### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA</td>
<td>IgA-class gliadin antibodies</td>
</tr>
<tr>
<td>ARA</td>
<td>IgA-class reticulin antibodies</td>
</tr>
<tr>
<td>AU</td>
<td>arbitrary units</td>
</tr>
<tr>
<td>CD</td>
<td>coeliac disease</td>
</tr>
<tr>
<td>Celikey tTG</td>
<td>a commercial test for immunoglobulin A class tissue transglutaminase antibodies (Celikey®, Phadia, Freiburg, Germany)</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>EMA</td>
<td>IgA-class endomysial antibodies</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay technique</td>
</tr>
<tr>
<td>ESPGAN</td>
<td>European Society for Paediatric Gastroenterology and Nutrition</td>
</tr>
<tr>
<td>Eu-tTG</td>
<td>a commercial test for immunoglobulin A class tissue transglutaminase antibodies (Eu-tTG® umana IgA, Eurospital S.p.A., Trieste, Italy)</td>
</tr>
<tr>
<td>GFD</td>
<td>gluten-free diet</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
</tr>
<tr>
<td>ICD</td>
<td>international classifications of diseases</td>
</tr>
<tr>
<td>IgA</td>
<td>immunoglobulin A</td>
</tr>
<tr>
<td>IELs</td>
<td>intraepithelial lymphocytes</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>RR</td>
<td>relative risk</td>
</tr>
<tr>
<td>SMR</td>
<td>standardized mortality ratio</td>
</tr>
<tr>
<td>tTG-ab</td>
<td>IgA-class tissue transglutaminase antibodies</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Coeliac disease is an autoimmune-mediated enteropathy with intestinal and extraintestinal manifestations triggered by the ingestion of gluten-containing grains, i.e. wheat, rye and barley, in genetically susceptible persons. As a predisposing factor roughly 90% of cases carry the human leukocyte antigen (HLA) DQ2 and most of the remainder the HLA DQ8 haplotype (Sollid et al. 1989, Polvi et al. 1996).

The clinical picture of coeliac disease has changed considerably since the 1970s, when a variety abdominal complaints such as steatorrhoea and abdominal distension as well as symptoms and signs of malabsorption such as weight loss evoked suspicion of the disease (Cooke and Holmes 1984). At that time coeliac disease was considered a rare condition affecting approximately 0.01% of Western populations (Greco et al. 1992). Following the introduction of serological tests for coeliac antibodies (Seah et al. 1971, Carswell and Ferguson 1972), preselection of patients for diagnostic small-bowel biopsy became possible. As a consequence, coeliac disease could be detected in patients with mild abdominal complaints and with a wide range of extraintestinal manifestations such as dermatitis herpetiformis, permanent teeth enamel defects, decreased bone mineral density and fractures as well as certain neurological disorders (Reunala et al. 1984, Aine et al. 1990, Hadjivassiliou et al. 1996, Mustalahti et al. 1999). However, the most serious complications associated with diagnosed coeliac disease were malignancies, especially lymphomas (Harris et al. 1967, Selby and Gallagher 1979, Holmes et al. 1989) and cases with the condition were also repeatedly shown to carry an increased risk of mortality (Logan et al. 1989, Cottone et al. 1999). Serological tests for coeliac autoantibodies further enabled the screening of risk groups such as first-degree relatives and type 1 diabetic patients independent of symptoms (Mäki et al. 1984, Mäki et al. 1991).

The widened understanding of the diversity of the condition and improved diagnostic facilities have resulted in a substantial increase in the incidence of the
diagnosed disease in Western countries (Collin et al. 1997a, Murray et al. 2003). In Finland as well as in North America, a ten-fold increase in incidence was reported over time. Over and above the increasing incidence figures, the first serological population screening studies in the early 1990s uncovered a number of previously undetected cases and unexpectedly high prevalence figures (Grodzinsky et al. 1992, Catassi et al. 1994, Johnston et al. 1997, Csizmadia et al. 1999). Undetected subjects were estimated to outnumber those with diagnosed disease by as much as 5-10:1 (Catassi et al. 1994, Csizmadia et al. 1999) and step by step coeliac disease was shown to have worldwide distribution affecting roughly 0.5-1% of populations on all continents, with only few exceptions (Catassi et al. 1999, Gomez et al. 2001, Hovell et al. 2001, Fasano et al. 2003, Tatar et al. 2004, Sood et al. 2006). The question arose, whether the increasing figures in coeliac disease were due solely to better diagnostics or whether they might reflect a true increase in disease frequency. In point of fact, the hypothesis here that the total prevalence of the disease, including both diagnosed and unrecognized cases, could have increased in the recent past, is also supported by rising frequencies of other autoimmune diseases such as type 1 diabetes and of allergic conditions (Woolcock and Peat 1997, Harjutsalo et al. 2008, Patterson et al. 2009).

As the finding of numerous unrecognized cases is relatively new, prognostic studies so far have been virtually limited to diagnosed cases. The approach has ultimately led to selection of patients, i.e. the data may not be applicable to the undetected condition. Increased risks of overall malignancies (Harris et al. 1967, Holmes et al. 1989, Askling et al. 2002) and mortality (Logan et al. 1989, Cottone et al. 1999, Viljamaa et al. 2006) have previously been reported in diagnosed cases, but the effect of the disease on the health of those with undetected condition is unclear as regards these more extreme outcomes. In the absence of evidence-based data as to whether it is beneficial to diagnose and treat coeliac disease in its early stages, it is crucial also to investigate the prognosis in the undetected section of the coeliac disease population.

In summary, the purpose of the present study was to assess any changes in the total prevalence of coeliac disease two decades apart and to describe the prognosis of undetected coeliac disease as regards more severe outcomes such as malignancies and mortality.
2. REVIEW OF THE LITERATURE

2.1 DEFINITION OF COELIAC DISEASE

Coeliac disease is a chronic autoimmune-mediated systemic disorder commonly presenting as enteropathy in genetically susceptible individuals (Green and Cellier 2007). The disease is triggered by cereal gluten from wheat, barley and rye and it is strongly associated with the HLA molecules DQ2 and DQ8. It is a common nutrient-related chronic disorder in the world, affecting roughly 0.5-1% of the populations of Europe, North and South America, Oceania, the Middle East, South Asia and North Africa (Catassi 2005). The current therapy for the condition is a life-long gluten-free diet.

2.2 CLINICAL PICTURE

2.2.1 Classical symptoms

Coeliac disease was first considered to be a serious intestinal disease of childhood, the majority of cases presenting in infancy (Cooke and Holmes 1984). Typical, so-called classical symptoms associated with the disease were steatorrhoea, abdominal distension and failure to thrive, but anorexia, vomiting, constipation, muscular wasting, rickets, irritability and extreme lethargy were by no means uncommon. The currently extremely rare clinical picture, “coeliac crisis”, with explosive diarrhoea, marked abdominal distension, dehydration, hypoproteinemia and hypotension, has been described in some cases (Cooke and Holmes 1984).

In older children different signs of malnutrition such as growth failure and anaemia were typical for the disease (Cooke and Holmes 1984). Following the introduction of gastroscopy and duodenal biopsy (Shiner 1957), it was soon recognized that coeliac disease may present at any age. Adults seemed to suffer
from symptoms similar to those in children, diarrhoea, weight loss and anaemia, but the mode of presentation was usually less acute (Cooke and Holmes 1984). Subsequently, mild or atypical gastrointestinal complaints such as abdominal discomfort, poor appetite, malaise, stomach growling and flatulence also came to be associated with coeliac disease (Logan et al. 1983, Mäki et al. 1988a).

Further, biochemical markers, i.e. anaemia, microcytosis, iron, folic acid, B12- and D-vitamin deficiencies, hypocalcaemia, hypoproteinaemia and transaminasaemia may also be suggestive of coeliac disease (Kemppainen et al. 1998, Volta et al. 1998, Catassi et al. 2007). A gluten-free diet has been found to ameliorate both gastrointestinal symptoms and nutritional status (Cooke and Holmes 1984, Kemppainen et al. 1998).

2.2.2 Extraintestinal symptoms and complications

Dermatitis herpetiformis, an itchy erythematous papulovesicular rash generally involving the extensor surfaces of the elbows, knees, buttocks and scalp, was connected to coeliac disease decades ago (Fry et al. 1973, Reunala et al. 1984). The condition has since been shown to be a gluten-sensitive disease with some degree of enteropathy, i.e. a coeliac disease of the skin (Reunala et al. 1984, Fry 2002). Further, the introduction of serological tests for coeliac antibodies in the 1970s (Seah et al. 1971, Carswell and Ferguson 1972) enabled screening of cases without classical symptoms, i.e. gastrointestinal complaints and malabsorption, and thus widened the understanding of the clinical manifestations of coeliac disease (Cooke and Holmes 1984, Mäki et al. 1988a, Collin et al. 1997a). Concomitant with a shift towards older age groups, a changing pattern of the clinical presentation towards milder and extraintestinal forms was reported (Logan et al. 1983, Mäki et al. 1988a). Currently, many coeliac disease cases are diagnosed by relatively mild digestive symptoms and roughly one in four by extraintestinal manifestations (Collin et al. 1997a). Although the reasons for the changed clinical picture are not fully known, it has been suggested to result from altered infant feeding practices and later onset of disease (Logan et al. 1983, Mäki et al. 1988a, Visakorpi 1996).

Since classification of different symptoms and illnesses into either extraintestinal manifestations or complications of coeliac disease is artificial, we show the
combined data in Table 1. Even though the strength of evidence of the connections of diverse symptoms and complications with coeliac disease may vary, it is clear that extraintestinal manifestations of the disorder are common and variable (Table 1).

**Table 1. Extraintestinal presentations and complications of coeliac disease (CD)**

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Data from the literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatitis herpetiformis</td>
<td>- Virtually all patients with dermatitis herpetiformis have some degree of enteropathy, roughly 15% of patients with CD have dermatitis herpetiformis, a gluten-free diet (GFD) heals both skin and enteropathy (Reunala et al. 1984, Fry 2002)</td>
</tr>
<tr>
<td>Neurological disorders</td>
<td>- 17% of adult patients with neurologic symptoms of unknown origin have been found to have biopsy-proven CD (Hadjivassiliou et al. 1996)</td>
</tr>
<tr>
<td></td>
<td>- Up to 49% of patients with CD have symptoms of peripheral neuropathy; the effect of a GFD is controversial (Cicarelli et al. 2003, Bushara 2005)</td>
</tr>
<tr>
<td></td>
<td>- 2-17% of patients with sporadic idiopathic cerebellar ataxia have biopsy-proven CD; the effect of a GFD has been variable (Hadjivassiliou et al. 1998, Bushara 2005)</td>
</tr>
<tr>
<td></td>
<td>- 1-3% of patients with epilepsy have CD, 4-6% of patients with CD have epilepsy, a rare epileptic condition with bilateral occipital cerebral calcification is especially associated with CD; the effect of GFD is unclear (Luostarinen et al. 2001, Bushara 2005)</td>
</tr>
<tr>
<td></td>
<td>- Association with headache and dementia is possible; influence of a GFD on symptoms needs to be confirmed (Collin et al. 1991, Luostarinen et al. 2001, Cicarelli et al. 2003)</td>
</tr>
<tr>
<td>Enamel defects and aphtous stomatitis</td>
<td>- 10-96% of patients with CD have systematic dental enamel defects in permanent dentition (Aine et al. 1990, Pastore et al. 2008)</td>
</tr>
<tr>
<td></td>
<td>- Roughly 5% of patients with aphtous stomatitis have CD, 10-41% of patients with CD have recurrent aphtous stomatitis; a GFD may be beneficial (Lähteenoja et al. 1998, Pastore et al. 2008)</td>
</tr>
<tr>
<td>Low bone mineral density and fractures</td>
<td>-CD cases evince decreased bone mineral density, which is improved by a GFD, the risk of fractures is increased by an average of 40% according to meta-analysis (Mustalahti et al. 1999, Davie et al. 2005, Olmos et al. 2008)</td>
</tr>
<tr>
<td>Fertility and pregnancy-related events</td>
<td>-CD may be associated with infertility in men and women, CD females have been shown to have a shorter reproductive period, number of miscarriages increased by 30%, the effect of a GFD is unclear (Farthing et al. 1982, Sher and Mayberry 1996, Tata et al. 2005) -Foetuses of untreated CD women have been shown to carry an excess risk of intrauterine growth retardation (Salvatore et al. 2007)</td>
</tr>
<tr>
<td>Psychiatric problems</td>
<td>-One third of patients with coeliac disease have psychiatric symptoms, an association with depression, anxiety and even psychosis has been suggested; the effect of a GFD is unclear (Pynnönen et al. 2004, Ludvigsson et al. 2007b, Ludvigsson et al. 2007e, Addolorato et al. 2008)</td>
</tr>
<tr>
<td>Joint symptoms</td>
<td>-Non-specific arthralgia and arthritis can also be manifestations of CD; the effect of a GFD is unclear (Mäki et al. 1988b, Lubrano et al. 1996)</td>
</tr>
<tr>
<td>Liver problems</td>
<td>-Abnormal liver biochemistry with elevated transaminases in approximately half of CD patients, this being reversible with GFD; CD is over presented in patients with chronic liver disease often autoimmune in origin; a GFD may be beneficial (Volta et al. 1998, Kaukinen et al. 2002b, Rubio-Tapia and Murray 2007)</td>
</tr>
<tr>
<td>Splenic hypofunction</td>
<td>-May be found in CD patients; a GFD seems to be beneficial (O'Grady et al. 1984, Corazza et al. 1999)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>-The risk of pancreatitis of any type was three-fold among CD patients hospitalized for any reason, the risk of chronic pancreatitis was especially emphasized, being 19-fold (Ludvigsson et al. 2007a)</td>
</tr>
<tr>
<td>Cardiac diseases</td>
<td>-Association with ischaemic diseases of the circulatory system as well as with cardiomyopathy, pericarditis and myocarditis is conflicting (Prati et al. 2002, West et al. 2004b, Elfstrom et al. 2007a, Ludvigsson et al. 2007c)</td>
</tr>
</tbody>
</table>

GFD=gluten-free diet
2.2.3 Associated conditions

The tendency of coeliac disease to run in families was already recognized in the 1960s (MacDonald et al. 1965). Since then, several studies have reported the prevalence of coeliac disease among the first-degree relatives of a proband to be around 10% (Mäki et al. 1991, Högberg et al. 2003).

A number of other associations with coeliac disease have been suggested (Cooke and Holmes 1984), but the majority of them seem to be coincidental, as screening studies since the 1990s have yielded prevalence figures much higher than previously assumed. However, the association with autoimmune disorders would appear to be strong, roughly 15-30% of coeliac disease patients having at least one associated autoimmune disorder compared to 1-11% in controls (Ventura et al. 1999, Sategna Guidetti et al. 2001, Viljamaa et al. 2005a, Guariso et al. 2007). The most widely recognized autoimmune disorders are type 1 diabetes, autoimmune thyroid disease with either hypo- or hyperthyroidism, and IgA deficiency (Table 2). Genetic diseases such as Down’s syndrome are also associated with coeliac disease (Table 2).

The effect of a gluten-free diet on the development of the associated autoimmune disorders remains inconclusive (Ventura et al. 1999, Sategna Guidetti et al. 2001, Viljamaa et al. 2005a, Guariso et al. 2007). Ventura and colleagues (1999) showed for the first time that the prevalence of autoimmune disorders increases with increasing age at diagnosis of coeliac disease. Age at diagnosis was thought to be a proxy measure of the duration of exposure to gluten. When the diagnosis had been made before the age of 2 years or over 10 years, the prevalence of autoimmune disorders was 5.1% and 23.6%, respectively (Ventura et al. 1999). This finding is supported by other studies in which increasing age at diagnosis of coeliac disease has been shown to be associated with autoimmune disorders, but on the other hand, for reasons unknown no association with the actual duration of gluten exposure was found in these studies (Sategna Guidetti et al. 2001, Viljamaa et al. 2005a). As to a gluten-free diet, adherence seems to reduce the risk of other clinical autoimmune diseases (Cosnes et al. 2008) but not the development of different autoantibodies (Guariso et al. 2007).
Table 2. Associated conditions in coeliac disease (CD).

<table>
<thead>
<tr>
<th>Associated condition</th>
<th>Strength of association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune thyroid disease</td>
<td>In 10-15% CD cases, 2-4% of patients with autoimmune thyroid disease have CD (Kuitunen et al. 1971, Hakanen et al. 2001, Collin et al. 2002a)</td>
</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>In 1-7% CD cases, approximately 4% of patients with type 1 diabetes have CD (Mäki et al. 1984, Collin et al. 2002a)</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>5-15% of Sjögren’s syndrome patients have CD (Iltanen et al. 1999, Szodoray et al. 2004)</td>
</tr>
<tr>
<td>Autoimmune liver disorders</td>
<td>3-7% of cases with either primary biliary chirrosis or autoimmune hepatitis have CD (Kingham and Parker 1998, Volta 2008)</td>
</tr>
<tr>
<td>Down’s syndrome</td>
<td>Roughly 5% of Down cases have CD (Bonamico et al. 2001b, Sanchez-Albisua et al. 2002)</td>
</tr>
<tr>
<td>Selective IgA deficiency</td>
<td>In approximately 2% CD cases, 10% of cases with selective IgA deficiency have CD (Mawhinney and Tomkin 1971, Savilahti et al. 1971, Cataldo et al. 1998, Korponay-Szabo et al. 2003)</td>
</tr>
<tr>
<td>Alopecia areata</td>
<td>Roughly 2% of cases with alopecia areata have CD (Corazza et al. 1995)</td>
</tr>
<tr>
<td>Addison’s disease</td>
<td>The risk in CD is at least five-fold (Reunala et al. 1987, Elfstrom et al. 2007b)</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>An association with CD has been suggested (Helin et al. 1983, Collin et al. 2002b)</td>
</tr>
<tr>
<td>Turner’s syndrome</td>
<td>An association with CD has been suggested (Bonamico et al. 1998, Frost et al. 2009)</td>
</tr>
</tbody>
</table>

IgA=immunoglobulin A

As coeliac disease cases with associated conditions are often apparently asymptomatic regarding coeliac disease, serological risk-group screening is used to find such cases (Green and Cellier 2007). According to the Finnish national recommendation risk groups should be extensively screened and the need to screen first-degree relatives as well as patients with IgA deficiency, type 1 diabetes, thyroid...
autoimmune disorders, Sjögren’s syndrome and alopecia areata is emphasized (Collin et al. 1997b). The reported benefits of screening followed by treatment are healing of potential symptoms, improved quality of life, increased bone mineral density, better metabolic control in type 1 diabetes and decreased amounts of thyroxin in hypothyroidism (Mustalahti et al. 1999, Mustalahti et al. 2002, Collin et al. 2002a). Screen-detected individuals have also been shown to have good compliance, bone mineral density and quality of life after long-term treatment (Viljamaa et al. 2005b). Approximately one fourth of coeliac disease cases are currently diagnosed as a consequence of risk-group screening or on the basis of findings in routine small-bowel biopsy without suspicion of coeliac disease (Collin et al. 1997a).

2.3 DIAGNOSIS

2.3.1 Diagnostic criteria for coeliac disease

The diagnosis of coeliac disease is currently based on both typical small-bowel biopsy findings (i.e. villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis) and a recovery of all symptoms on a gluten-free diet, as stated by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) (Walker-Smith et al. 1990). The finding of coeliac autoantibodies at time of diagnosis and their disappearance on a gluten-free diet supports the diagnosis. A second biopsy for confirmation of histological recovery is currently mandatory only in asymptomatic cases but is still frequently carried out in adults (Walker-Smith et al. 1990). Nor is a gluten challenge with the third biopsy included in the present diagnostic criteria, but may still be helpful when there is doubt as to the initial diagnosis or the adequacy of the clinical response to a gluten-free diet (Walker-Smith et al. 1990). Thus, the three small-bowel biopsies previously recommended in the diagnosis of coeliac disease (Meeuwisse 1970) are currently seldom needed.

New diagnostic criteria have been called for in consequence of widened understanding of gluten sensitivity (Ferguson et al. 1993, Kaukinen et al. 2001). It is well established that small-intestinal mucosal damage develops gradually over years or even decades, beginning from normal morphology and continuing through
inflammation to villous atrophy and crypt hyperplasia (Marsh 1992). Cases with early mild mucosal changes not serious enough to fulfil the current diagnostic criteria have repeatedly been shown to be gluten-sensitive or to develop manifest mucosal lesion in later life (Egan-Mitchell et al. 1981, Mäki et al. 1990, Collin et al. 1993, Salmi et al. 2006, Kurppa et al. 2009). On the other hand, it has been suggested that cases with high coeliac autoantibody values in serological screening might not need confirmation of diagnosis by small-bowel biopsy (Valdimarsson et al. 1996, Hill and Holmes 2008). Consensus is needed on the diagnostic criteria for cases with mild mucosal changes or high antibody levels.

2.3.2 Serology

Non-invasive tools, i.e. measurement of coeliac antibodies, have for decades facilitated preselection of patients for small-bowel biopsy. The most sensitive antibody tests for the diagnosis of coeliac disease are of the immunoglobulin A (IgA) class, and tests for anti-reticulin and anti-gliadin antibodies (AGA) were first introduced in the early 1970s (Seah et al. 1971, Carswell and Ferguson 1972). Anti-reticulin antibodies were defined by an indirect immunofluorescence method using rat tissues as antigens and AGA correspondingly by an enzyme-linked immunosorbent assay technique (ELISA) using wheat gliadin as antigen.

A decade later a new era of serological testing of coeliac disease opened up, when highly valid IgA-class endomysial antibodies (EMA) were detected by an indirect immunofluorescence method using monkey oesophagus as antigen (Chorzelski et al. 1983). However, the cost involved and ethical issues with the substrate inspired the development of a currently widely used test for EMA with human umbilical cord as a new substrate (Ladinser et al. 1994). The high sensitivity and specificity of the new test in untreated coeliac disease have been repeatedly evidenced independent of the age of patients (Table 3). Two systematic reviews yielded 90% and 92% as the pooled estimates for the sensitivity of the EMA test in adults, and the corresponding figures for specificity were 100% in both reports (Rostom et al. 2005, Lewis and Scott 2006). Small-bowel villous atrophy has been used as the golden standard in these reports.
Table 3. Sensitivity and specificity of human umbilical cord-based IgA-class endomysial antibodies as against villous atrophy in small-bowel biopsy in untreated coeliac disease.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patients¹, n</th>
<th>Controls¹, n</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>adults</td>
<td>60</td>
<td>200</td>
<td>95</td>
<td>100</td>
<td>Volta et al. 1995</td>
</tr>
<tr>
<td>adults</td>
<td>136</td>
<td>207</td>
<td>93</td>
<td>100</td>
<td>Sulkaneen et al. 1998</td>
</tr>
<tr>
<td>adults</td>
<td>27</td>
<td>65</td>
<td>96</td>
<td>98</td>
<td>Lock et al. 1999</td>
</tr>
<tr>
<td>adults</td>
<td>39</td>
<td>61</td>
<td>100</td>
<td>100</td>
<td>Biagi et al. 1999</td>
</tr>
<tr>
<td>adults</td>
<td>114</td>
<td>65</td>
<td>87</td>
<td>100</td>
<td>Dahele et al. 2001</td>
</tr>
<tr>
<td>adults</td>
<td>51</td>
<td>36</td>
<td>80</td>
<td>100</td>
<td>Raivio et al. 2007</td>
</tr>
<tr>
<td>children</td>
<td>50</td>
<td>25</td>
<td>94</td>
<td>100</td>
<td>Bottaro et al. 1997</td>
</tr>
<tr>
<td>children</td>
<td>53</td>
<td>114</td>
<td>94</td>
<td>100</td>
<td>Kolho and Savilahti 1997</td>
</tr>
<tr>
<td>children</td>
<td>24</td>
<td>71</td>
<td>46</td>
<td>96</td>
<td>Russo et al. 1999</td>
</tr>
<tr>
<td>mixed</td>
<td>30</td>
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<td>100</td>
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<tr>
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<td>20</td>
<td>93</td>
<td>100</td>
<td>Sblattero et al. 2000</td>
</tr>
<tr>
<td>mixed</td>
<td>103</td>
<td>89</td>
<td>90</td>
<td>99</td>
<td>Stern et al. 2000</td>
</tr>
<tr>
<td>mixed</td>
<td>126</td>
<td>106</td>
<td>89</td>
<td>98</td>
<td>Collin et al. 2005</td>
</tr>
<tr>
<td>mixed</td>
<td>143</td>
<td>74</td>
<td>96</td>
<td>100</td>
<td>Mankai et al. 2005</td>
</tr>
</tbody>
</table>

¹ Coeliac disease diagnosed or excluded by small-bowel mucosal biopsy

Recognition of tissue transglutaminase as an autoantigen in coeliac disease (Dieterich et al. 1997) allowed the development of an automated ELISA for IgA-class tissue transglutaminase antibodies (tTG-ab) (Dieterich et al. 1998, Sulkaneen et al. 1998). The test for tTG-ab penetrated the market quickly, as it was more objective, less expensive and labour-intensive than the test for EMA. However, since the first test introduced using guinea-pig liver tissue transglutaminase as antigen could not outperform the test for EMA in validity, the test using human tissue transglutaminase (from red blood cells or recombinant based) was developed and is nowadays widely used (Fabiani et al. 2004, Collin et al. 2005, Lewis and Scott 2006). Good accuracy of the human recombinant-based test for tTG-ab as against villous atrophy in small-bowel biopsy in coeliac disease is evidenced in Table 4. According to the two above-mentioned systematic review articles the pooled sensitivities of the test have been 98% and 100% in adults, and specificities 98% and 97%, respectively (Rostom et al. 2005, Lewis and Scott 2006). Regardless of the reported high specificity of the human recombinant-based test for tTG-ab in general, there are reports of high false positivity rates in patients with chronic liver,
inflammatory bowel or end-stage heart disease (Peracchi et al. 2002, Lo Iacono et al. 2005, Villalta et al. 2005, Tursi 2006) and thus, the purity of human recombinant tissue transglutaminase has been emphasized (Clemente et al. 2002, Bizzaro et al. 2006, Sardy et al. 2007).

Table 4. Sensitivity and specificity of human recombinant-based IgA-class tissue transglutaminase antibodies as against villous atrophy in small-bowel biopsy in untreated coeliac disease.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patients, n</th>
<th>Controls, n</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>adults</td>
<td>21</td>
<td>128</td>
<td>95</td>
<td>100</td>
<td>Gillett and Freeman 2000</td>
</tr>
<tr>
<td>adults</td>
<td>110</td>
<td>26</td>
<td>100</td>
<td>100</td>
<td>Picarelli et al. 2001</td>
</tr>
<tr>
<td>adults</td>
<td>24</td>
<td>183</td>
<td>100</td>
<td>97</td>
<td>Carroccio et al. 2002</td>
</tr>
<tr>
<td>children</td>
<td>42</td>
<td>28</td>
<td>95</td>
<td>100</td>
<td>Vitoria et al. 2001</td>
</tr>
<tr>
<td>children</td>
<td>62</td>
<td>56</td>
<td>100</td>
<td>100</td>
<td>Bonamico et al. 2001a</td>
</tr>
<tr>
<td>children</td>
<td>52</td>
<td>49</td>
<td>96</td>
<td>100</td>
<td>Wolters et al. 2002</td>
</tr>
<tr>
<td>children</td>
<td>32</td>
<td>83</td>
<td>93</td>
<td>90</td>
<td>Leach et al. 2008</td>
</tr>
<tr>
<td>mixed</td>
<td>55</td>
<td>53</td>
<td>98</td>
<td>98</td>
<td>Sardy et al. 1999</td>
</tr>
<tr>
<td>mixed</td>
<td>70</td>
<td>196</td>
<td>93</td>
<td>99</td>
<td>Baldas et al. 2000</td>
</tr>
<tr>
<td>mixed</td>
<td>65</td>
<td>170</td>
<td>91</td>
<td>99</td>
<td>Sblattero et al. 2000</td>
</tr>
<tr>
<td>mixed</td>
<td>208</td>
<td>157</td>
<td>96</td>
<td>99</td>
<td>Burgin-Wolff et al. 2002</td>
</tr>
<tr>
<td>mixed</td>
<td>250</td>
<td>176</td>
<td>91</td>
<td>96</td>
<td>Tesei et al. 2003</td>
</tr>
<tr>
<td>mixed</td>
<td>61</td>
<td>64</td>
<td>98</td>
<td>97</td>
<td>Llorente et al. 2004</td>
</tr>
<tr>
<td>mixed</td>
<td>126</td>
<td>106</td>
<td>94</td>
<td>99</td>
<td>Collin et al. 2005</td>
</tr>
</tbody>
</table>

1 Coeliac disease diagnosed or excluded by small-bowel mucosal biopsy

The studies focusing on the validity of coeliac autoantibodies have also come in for criticism for other reasons (Rostom et al. 2005). Study settings with a high prevalence of coeliac disease yield higher positive predictive values compared to the situation in clinical practice. In addition, different inclusion and exclusion criteria for coeliac disease patients and controls may have biased figures for sensitivity and specificity. Notwithstanding the criticism for tests for EMA and tTG-ab, they are currently considered the best means of screening for coeliac disease and they have also been shown to correlate clearly with carriage of HLA DQ2 and DQ8 (Mäki et al. 2003).
In addition, the possibility of IgA deficiency should be kept in mind in serological screening and total IgA measured if the level of IgA-class coeliac autoantibodies is very low (Mawhinney and Tomkin 1971, Savilahti et al. 1971, Cataldo et al. 1998, Korponay-Szabo et al. 2003). In the case of IgA deficiency, tests for IgG-class endomysial or tissue transglutaminase antibodies should still be performed (Cataldo et al. 1998, Korponay-Szabo et al. 2003).

Rapid tests for tTG-ab using a sample of fingertip blood have also been developed in recent years to make testing more simple and quick (Korponay-Szabo et al. 2005, Raivio et al. 2006, Korponay-Szabo et al. 2007). Furthermore, new-generation antibodies to deamidated gliadin peptides have emerged as a promising addition in diagnostics (Kaukinen et al. 2007a).

2.3.3 Small-bowel mucosal biopsy

Villous atrophy and crypt hyperplasia characteristic for coeliac disease was first described in operative specimens of the jejunum (Paulley 1954) and subsequently by peroral biopsies (Sakula and Shiner 1957). As the spectrum of gluten sensitivity was recognized, Marsh and colleagues separated three main groups of mucosal changes termed infiltrative (type 1), hyperplastic (type 2) and destructive (type 3) (Marsh 1992). In the Marsh 1 lesion, villi remain unaltered but the epithelium is markedly infiltrated by intraepithelial lymphocytes (IELs), i.e. more than 40 per 100 enterocytes. In the hyperplastic Marsh 2 lesion there is an increased number of IELs with enlarged crypts. The most serious alteration is termed destructive or Marsh type 3 lesion, where villi are absent or rudimentary. The Marsh type 3 lesion was later modified into three different categories: 3a (mild villous atrophy), 3b (marked villous atrophy) and 3c (total villous atrophy) (Oberhuber et al. 1999). Continuous morphological measurements such as the villous height- crypt depth ratio and the number of IELs are also used in diagnostics (Ferguson and Murray 1971, Fry et al. 1972, Salmi et al. 2009).

To avoid difficulties in interpretation biopsies should be done distal to the duodenal bulb and an adequate number of biopsies (at least four samples) should also be taken (Green and Cellier 2007). This is important in ensuring sufficient well-orientated samples for interpretation of crypt to villous ratio and not missing
possible patchy lesions. Macroscopic changes in endoscopy associated with coeliac disease, i.e. absent or reduced duodenal folds, scalloping of mucosal folds or a mosaic appearance of the mucosa are not specific or sensitive for coeliac disease and thus, sampling of the small-bowel mucosa should be performed in all patients (Green and Cellier 2007).

At the moment, any patient with classical symptoms, i.e. chronic diarrhoea, weight loss or iron deficiency, should undergo a duodenal biopsy independent of serologic test result. Conversely, a duodenal biopsy is mainly recommended for patients with atypical or mild symptoms only if they yield a positive test result for coeliac autoantibodies (Mäki et al. 1988a, Collin et al. 1997a).

2.3.4 Diagnosis of dermatitis herpetiformis

Diagnosis of dermatitis herpetiformis is based on granular IgA in dermal papillary tips in perilesional skin by direct immunofluorescence examination (Fry 2002). Approximately 70-90% of patients with dermatitis herpetiformis have villous atrophy of the small-bowel mucosa and the remainder evince minor changes with increased numbers of IELs (Reunala et al. 1984, Fry 2002).

2.3.5 Latent and potential coeliac disease

Any subject who will develop histologically confirmed coeliac disease in the future or who has had the disease in the past, but at the time of investigation has normal small-bowel mucosa on a gluten-containing diet, is deemed to have latent coeliac disease (Ferguson et al. 1993). The definition of potential coeliac disease partly overlaps with the definition of latent coeliac disease. Potential coeliac disease refers to cases with HLA-predisposing genotype who have normal small-bowel mucosal architecture on a gluten-containing diet but who betray various immunological abnormalities as a mark of possible coeliac disease (Ferguson et al. 1993).

The abnormalities in latent or potential disease include coeliac autoantibodies (in sera, intestinal fluids or mucosa) and increased numbers of IELs, especially those bearing the γδ T cell receptor (Ferguson et al. 1993, Salmi et al. 2006). Cases with any of these markers are at an increased risk of developing a typical coeliac disease
enteropathy later in life (Salmi et al. 2006). The proportion of coeliac autoantibody-positive cases developing villous atrophy in surveillance has varied between 30 and 50% depending on the study and follow-up time (Collin et al. 1993, Kaukinen et al. 1998, Salmi et al. 2006). In some study settings, dermatitis herpetiformis with mild small-bowel mucosal changes has been used as a model of latent coeliac disease (Fry et al. 1972).

2.3.6 Differential diagnosis

Inflammation and architectural changes of in the small-bowel mucosa should also prompt consideration of non-coeliac states especially in seronegative cases. These include: a) specific intestinal illnesses (Crohn’s disease, autoimmune enteropathy, bacterial overgrowth syndrome, collagenous sprue, tropical sprue, eosinophilic gastroenteritis, Zollinger–Ellison syndrome, intestinal lymphoma) b) intolerance of or allergy to foods other than gluten (e.g., milk, soy) c) intestinal infections (e.g. rotavirus, giardia, cryptosporidium, tuberculosis) d) immunodeficiency states e) iatrogenic aetiology (radiotherapy, drugs) (Green and Cellier 2007).

2.4 GENETICS

Even though environmental factors, especially gluten, play a major role in the pathogenesis of coeliac disease, susceptibility to the condition is clearly inheritable. A familial aggregation is found in roughly 10% of coeliac disease patients (Mäki et al. 1991, Högberg et al. 2003) and the concordance rate of over 80% in monozygotic twins further underlines the role of genes in the development of coeliac disease (Greco et al. 2002, Nistico et al. 2006).

At present it is known that the disease is strongly associated with genes of the major histocompatibility complex (MHC) and especially the human leucocyte antigen (HLA) class II DQ genes located on the short arm of chromosome six (6p21) (Sollid et al. 1989, Polvi et al. 1996). These susceptibility genes encode glycoproteins, HLA DQ2 and DQ8 heterodimers, on the membranes of cells involved in immune responses and the main function of these heterodimers is to present peptide antigens to T lymphocytes. Roughly 90% of coeliac disease patients
carry the HLA DQ2 heterodimer, this being composed of α and β chains and encoded by the alleles DQA1*05 (α) and DQB1*02 (β). These alleles can be inherited in cis, that is on one chromosome (DR3 or DR17 haplotype) or in trans, each allele in different chromosomes (DR5/7 or DR11/12 haplotype) (Sollid et al. 1989). Almost all HLA DQ2-negative coeliac disease patients carry the HLA DQ8 heterodimer encoded by the alleles DQA1*0301 (α chain) and DQB1*0302 (β chain) (DR4 haplotype) (Karell et al. 2003). Further, a few patients carry solely either the α or the β chain of DQ2 heterodimer (Karell et al. 2003). Coeliac disease patients not carrying any of these HLA susceptibility genes are a rarity and absence of risk alleles is thus also of clinical significance in suspicion of the condition (Wolters and Wijmenga 2008). The proportion of individuals with DR3-DQ2 haplotype varies substantially according to geographical area (Fasano and Catassi 2001). It is as much as 20 to 30% in North Africa and South-West Asia, 10 to 15% in Europe and North America and no more than 0 to 5% in the Far East, South Africa and South America.

HLA DQ2 and DQ8 are the most important identified genetic risk factors for coeliac disease (locus named COELIAC1), but they would appear not to be sufficient to predispose to coeliac disease, as these risk alleles are identified not only in coeliac disease but also in 30 to 40% of the general population (Sollid et al. 1989, Polvi et al. 1996). Candidate gene regions named COELIAC2, COELIAC3 and COELIAC4 loci have been recognized by genetic linkage studies (Wolters and Wijmenga 2008). The COELIAC2 locus on chromosome 5q31-33 contains a cytokine gene cluster and could take part in immune regulation and inflammation. The COELIAC3 locus on chromosome 2q33 contains the T lymphocyte regulatory genes and the COELIAC4 locus on chromosome 19p13 the myosin IXB gene which encodes the myosin molecule. It has been hypothesized that a genetic variant of the myosin IXB gene might lead to an impaired intestinal barrier. Recently, genome-wide association studies have further uncovered eight new genomic regions associated with coeliac disease, for example on chromosome 4q27 and 3p21 (van Heel et al. 2007, Hunt et al. 2008). Interestingly, the majority of these regions contain genes which have immune functions. It seems likely that many non-HLA genes contribute to the pathogenesis of coeliac disease while the contribution of a single predisposing non-HLA gene might be modest. It has been estimated that the known non-HLA risk loci for coeliac disease account for only 3-4% of disease
genetic heritability, with the HLA genes accounting for a further 30% (Hunt and van Heel 2009).

### 2.5 PATHOGENESIS

Even though the pathogenesis of coeliac disease is yet not fully understood, genes are clearly not sufficient to account for the development of coeliac disease; environmental and immunogenic factors are equally vital in the pathogenesis. Dietary exposure to gluten has a central role in triggering the mucosal damage (Shan et al. 2002). The term “gluten” refers to toxic or immunogenic peptides in the storage proteins of wheat, barley and rye. These glutamine- and proline-rich peptides are called gliadins in wheat, hordeins in barley and secalins in rye. The peptides resistant to degradation of gastrointestinal proteases (Shan et al. 2002) are able to activate both innate and adaptive immune responses in the intestinal mucosa (Maiuri et al. 2000, Gianfrani et al. 2005).

Increased permeability of the intestine gives access to the gliadin peptides through the epithelium. It has been hypothesized that viral infections or genetic changes could be in the background (Fasano et al. 2000, Koskinen et al. 2008, Wolters and Wijmenga 2008). The gluten peptides use both paracellular and transepithelial passages to reach the lamina propria (Fasano et al. 2000, Koskinen et al. 2008, Wolters and Wijmenga 2008). Toxic gluten peptides induce upregulation of interleukin 15 in the lamina propria mononuclear and dendritic cells as an unspecific innate immune response (Maiuri et al. 2000). Interleukin 15, again, induces proliferation of IELs and upregulation of enterocyte MICA molecules as well as the receptor of the same molecules in T cells, which might take part in enterocyte apoptosis and villous atrophy in coeliac disease (Mention et al. 2003, Hue et al. 2004).

Several peptides derived from various gluten proteins, including α- and γ-gliadins and even glutenins, activate the adaptive immune response in genetically susceptible persons carrying either HLA DQ2 or DQ8 molecules (Shan et al. 2002, Gianfrani et al. 2005). First, glutamic residues of the peptides are deamidated by the enzyme tissue transglutaminase to negatively charged glutamic acid in order to facilitate their binding to the peptic groove of HLA DQ2 or DQ8 molecules on antigen
presenting cells (Molberg et al. 1998). The binding in turn activates two types of CD4+ T helper cell responses, designated Th1 and Th2 (Molberg et al. 1998). The Th1 response is characterized by release of interferon γ and tumour necrosis factor α (Nilsen et al. 1995), which induce secretion of matrix metalloproteinases from intestinal fibroblasts, being thus possibly responsible for matrix breakdown and the small-bowel mucosal lesion in coeliac disease (Pender et al. 1997). The Th2 response in turn is characterized by activation of B-cell antibody production against gluten and tissue transglutaminase (Gianfrani et al. 2005). The role of the antibodies in the pathogenesis remains equivocal. However, it has been suggested that they might also indirectly contribute to increasing proliferation and decreasing differentiation of epithelial cells (Halttunen and Mäki 1999, Lindfors et al. 2009). Be this as it may, both innate and adaptive immune responses induce morphological changes typical for coeliac disease, i.e. villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis.

2.6 EPIEMIOLOGY

2.6.1 Prevalence of coeliac disease and dermatitis herpetiformis

Our conception of the epidemiology of coeliac disease has changed substantially during the last decades. Even in the late 1970s coeliac disease was still considered a rare disorder affecting approximately 1 per 1,000 (0.1%) individuals mostly of European origin (Greco et al. 1992). However, based on a substantial number of screening studies (Tables 5 and 6) it is currently considered to constitute a prevalent disorder affecting roughly 0.5-1% of the general population in many countries worldwide. As both genetic (HLA and non-HLA genes) and environmental factors (wheat consumption) are crucial in the pathogenesis of coeliac disease, the distribution of these two factors seems to identify the world areas-at-risk (Fasano and Catassi 2001). Thus, the disease is frequent in Europe, North and South America, Australia, South-West Asia and North Africa, where the DR3-DQ2 haplotype and high consumption of wheat are present (Fasano and Catassi 2001). In contrast, coeliac disease is extremely rare in the Far East and most probably also in sub-Saharan Africa, where wheat foods are not staple foods and the coeliac disease-
predisposing HLA haplotype seems to be rare (Fasano and Catassi 2001, Accomando and Cataldo 2004).

The screen-detected prevalence of coeliac disease in Europe is very high, ranging between 0.2 and 1.9% of the general population as defined by small-bowel biopsy or EMA (Table 5). So far, a prevalence of EMA positivity over 1% has been reported from Finland, Sweden, the United Kingdom (UK), the Netherlands, Spain, Italy and Hungary. Serological screening studies carried out in areas of European ancestry, i.e. North and South America and Oceania have yielded prevalence figures comparable to Europe in the recent past (Table 6). It is noteworthy that in less-developed countries coeliac subjects still often suffer from classical severe symptoms, which are nowadays rare in developed countries (Rawashdeh et al. 1996, Matek et al. 2000).

During the last few years several studies have also suggested coeliac disease to be common in South West Asia, as a high prevalence of the disease has been reported in patients with chronic diarrhoea and type 1 diabetes in that area (Rawashdeh et al. 1996, Al-Ashwal et al. 2003). Population screening studies have been carried out in Iran, Turkey and Israel and prevalence figures comparable to those in Europe have been shown (Table 6). In other parts of Asia data on coeliac disease prevalence in the general population is scarce, as there is only one study from India and two separate studies from the west part of Russia (Table 6). As the consumption of wheat is high in these areas, a high number of unrecognized coeliac disease cases may exist (Fasano and Catassi 2001). In contrast, only sporadic coeliac disease cases have been found in the Far East (e.g. China, Japan) (Freeman 2003, Jiang et al. 2009).

A high frequency of coeliac disease in North African Arab populations has been uncovered by screening studies of general populations and risk groups in very recent years (Table 6). However, there is only one study of the prevalence of coeliac autoantibodies in other parts of Africa: the population of Burkina Faso was found to be seronegative for both EMA and tTG-ab (Table 6). In addition, only a few sporadic clinically diagnosed coeliac disease cases have been reported in Black populations (immigrants) (Accomando and Cataldo 2004).
Table 5. Seroprevalence and biopsy-proven prevalence of screen-detected coeliac disease (CD) in Europe. The total prevalence, including both screen-detected cases and earlier diagnosed patients, is also given when available.

<table>
<thead>
<tr>
<th>Place of study</th>
<th>References</th>
<th>Participants, N</th>
<th>Age group</th>
<th>Seroprevalence (%)</th>
<th>Biopsy-proven prevalence (%)</th>
<th>Total prevalence of CD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tTG-ab</td>
<td>EMA</td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>Edlinger-Horvat et al. 2005</td>
<td>7,660 (^2)</td>
<td>adults</td>
<td>0.4</td>
<td>0.2 (^3)</td>
<td>ND</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Vancikova et al. 2002</td>
<td>1,312 (^4)</td>
<td>adults</td>
<td>7.0</td>
<td>0.5 (^3)</td>
<td>ND</td>
</tr>
<tr>
<td>Denmark</td>
<td>Weile et al. 1996</td>
<td>1,573 (^4)</td>
<td>adults</td>
<td>ND</td>
<td>0.2 (^3)</td>
<td>ND</td>
</tr>
<tr>
<td>Estonia</td>
<td>Ress et al. 2007</td>
<td>1,160</td>
<td>children</td>
<td>0.4</td>
<td>ND</td>
<td>0.3</td>
</tr>
<tr>
<td>Finland</td>
<td>Kolho et al. 1998</td>
<td>1,070 (^5)</td>
<td>adults</td>
<td>ND</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Mäki et al. 2003</td>
<td>3,654</td>
<td>children</td>
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<td>1.4</td>
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<td>children</td>
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<td>0.2</td>
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<td></td>
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<td>adults</td>
<td>ND</td>
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<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Roka et al. 2007</td>
<td>4,633</td>
<td>adults</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Greece</td>
<td>Roka et al. 2007</td>
<td>2,230</td>
<td>adults</td>
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<td>0.2 (^3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Hungary</td>
<td>Korponay-Szabo et al. 2007</td>
<td>2,676 (^6)</td>
<td>children</td>
<td>1.0</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Italy</td>
<td>Catassi et al. 1994</td>
<td>3,351</td>
<td>children</td>
<td>ND</td>
<td>0.3 (^3)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Volta et al. 2001</td>
<td>3,483</td>
<td>mixed</td>
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<td>0.5</td>
</tr>
<tr>
<td>Country</td>
<td>Study</td>
<td>Sample Size</td>
<td>Age Group</td>
<td>Prevalence</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>------------</td>
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<td>-------------</td>
<td>---------------</td>
<td>------------</td>
<td>-------------</td>
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</tr>
<tr>
<td>Netherlands</td>
<td>Tommasini et al. 2004</td>
<td>3,188</td>
<td>children</td>
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<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Csizmadia et al. 1999</td>
<td>6,127</td>
<td>children</td>
<td>ND</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Rostami et al. 1999</td>
<td>1,000</td>
<td>adults</td>
<td>ND</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Norway</td>
<td>Hovdenak et al. 1999</td>
<td>2,096</td>
<td>adults</td>
<td>ND</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Portugal</td>
<td>Antunes 2002</td>
<td>536</td>
<td>children</td>
<td>2.1</td>
<td>0.7</td>
<td>0.6</td>
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<tr>
<td>Spain</td>
<td>Riestra et al. 2000</td>
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<td>ND</td>
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<td>0.2</td>
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<tr>
<td></td>
<td>Castano et al. 2004</td>
<td>484</td>
<td>children</td>
<td>2.1 8</td>
<td>1.3 3</td>
<td>1.4</td>
</tr>
<tr>
<td>Sweden</td>
<td>Ivarsson et al. 1999</td>
<td>1,894</td>
<td>adults</td>
<td>ND</td>
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<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Carlsson et al. 2001</td>
<td>690</td>
<td>children</td>
<td>ND</td>
<td>1.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Rutz et al. 2002</td>
<td>1,450</td>
<td>children</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>UK</td>
<td>Johnston et al. 1997</td>
<td>1,823</td>
<td>adults</td>
<td>ND</td>
<td>ND</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>West et al. 2003</td>
<td>7,550</td>
<td>adults</td>
<td>ND</td>
<td>1.2</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Bingley et al. 2004</td>
<td>5,470</td>
<td>children</td>
<td>2.4</td>
<td>1.0 3</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Notes:**
1. The prevalence the authors reported; not always biopsy-proven.
2. Males attending compulsory medical examination before military service.
3. The test was carried out only in the second step of screening algorithm.
5. Persons attending for blood sampling because of a routine examination or suspicion of some disorder other than coeliac disease.
6. Preschool- or schoolchildren.
7. Volunteers from the general population.
8. A test for IgG-class tissue transglutaminase antibody was used.
Table 6. Seroprevalence and biopsy-proven prevalence of screen-detected coeliac disease (CD) outside Europe. The total prevalence, including both screen-detected cases and earlier diagnosed patients, is also given when available.

<table>
<thead>
<tr>
<th>Place of study</th>
<th>References</th>
<th>Participants, N</th>
<th>Age group</th>
<th>Seroprevalence (%)</th>
<th>Biopsy-proven prevalence (%)</th>
<th>Total prevalence of CD ¹ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tTG-ab</td>
<td>EMA</td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mexico</td>
<td>Remes-Troche et al. 2006</td>
<td>1,009 ²</td>
<td>adults</td>
<td>2.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>USA</td>
<td>Fasano et al. 2003</td>
<td>4,126 ³</td>
<td>mixed</td>
<td>0.8³</td>
<td>0.8</td>
<td>ND</td>
</tr>
<tr>
<td>USA</td>
<td>Neri et al. 2004</td>
<td>2,000</td>
<td>adults</td>
<td>1.5</td>
<td>0.8³</td>
<td>ND</td>
</tr>
<tr>
<td>South America</td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Argentina</td>
<td>Gomez et al. 2001</td>
<td>2,000 ⁵</td>
<td>adults</td>
<td>ND</td>
<td>0.5³</td>
<td>0.6</td>
</tr>
<tr>
<td>Brazil</td>
<td>Pratesi et al. 2003</td>
<td>4,405 ⁶</td>
<td>mixed</td>
<td>ND</td>
<td>0.4</td>
<td>0.2</td>
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<tr>
<td>Brazil</td>
<td>Pereira et al. 2006</td>
<td>2,086 ²</td>
<td>adults</td>
<td>0.3</td>
<td>0.3³</td>
<td>0.1</td>
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<tr>
<td>Oceania</td>
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<td></td>
<td>ND</td>
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<td>0.2</td>
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<td>Australia</td>
<td>Hovell et al. 2001</td>
<td>3,011</td>
<td>adults</td>
<td>ND</td>
<td>0.3</td>
<td>0.2</td>
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<tr>
<td>New Zealand</td>
<td>Cook et al. 2000</td>
<td>1,064</td>
<td>adults</td>
<td>ND</td>
<td>0.9</td>
<td>0.9</td>
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<td>Asia</td>
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<td></td>
<td></td>
<td>ND</td>
<td>0.3</td>
<td>ND</td>
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<tr>
<td>India</td>
<td>Sood et al. 2006</td>
<td>4,347</td>
<td>children</td>
<td>0.5⁷</td>
<td>ND</td>
<td>0.3</td>
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<tr>
<td>Location</td>
<td>Study Authors</td>
<td>Sample Size (N)</td>
<td>Age Group</td>
<td>Prevalence (IgA-class EMA)</td>
<td>Prevalence (IgA-class tTG-ab)</td>
<td>Notes</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>Iran</td>
<td>Shahbazkhani et al. 2003</td>
<td>2,000</td>
<td>adults</td>
<td>ND</td>
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<td>Akbari et al. 2006</td>
<td>2,799</td>
<td>adults</td>
<td>1.0</td>
<td>0.2</td>
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<td>Israel</td>
<td>Shamir et al. 2002</td>
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<td>adults</td>
<td>ND</td>
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<td>0.1</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Russia</td>
<td>Stroikova et al. 2006</td>
<td>1,740</td>
<td>adults</td>
<td>ND</td>
<td>2.4</td>
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<td></td>
<td>Kondrashova et al. 2008</td>
<td>1,988</td>
<td>children</td>
<td>0.6</td>
<td>0.5</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>Tatar et al. 2004</td>
<td>2,000</td>
<td>adults</td>
<td>ND</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Ertekin et al. 2005</td>
<td>1,263</td>
<td>children</td>
<td>0.9</td>
<td>0.6</td>
<td></td>
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<tr>
<td>Africa</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Algeria</td>
<td>Catassi et al. 1999</td>
<td>989</td>
<td>children</td>
<td>ND</td>
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<tr>
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<td>ND</td>
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<tr>
<td>Egypt</td>
<td>Abu-Zekry et al. 2008</td>
<td>1,500</td>
<td>children</td>
<td>0.9</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Tunisia</td>
<td>Bdioui et al. 2006</td>
<td>1,418</td>
<td>adults</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ben Hariz et al. 2007</td>
<td>6,284</td>
<td>children</td>
<td>1.4</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

**EMA**=Test for IgA-class endomysial antibodies, **tTG-ab**=Test for IgA-class tissue transglutaminase antibodies, **ND**=Not defined, **USA**=United States of America

1 The prevalence the authors reported, which was not always biopsy-proven
2 Blood donors
3 The test was carried out only in the second step of screening algorithm
4 Blood donors, school children and patients seen in outpatient clinics for routine checkups
5 Couples attending an obligatory prenuptial examination
6 Patients seen in outpatient clinics for routine checkups
7 The test for tTG-ab was carried out only if there was suspicion of coeliac disease in clinical assessment
8 Children from four different villages
9 General paediatric population with conditions unrelated to coeliac disease
10 Schoolchildren
As to the clinical prevalence of coeliac disease, this consists in the prevalence of patients found by health care professionals, thus differing from the above-mentioned screen-detected prevalence. The clinical prevalence has been increasing in Western countries since the 1960s, especially in adults, and a female predominance of roughly 2:1 has also been repeatedly shown (Logan et al. 1983, Murray et al. 2003, Collin et al. 2007). For example, the prevalence of coeliac disease increased ten-fold in 1975-1994 (Collin et al. 1997a). The increased figures have been thought to be due to better awareness of the multifaceted clinical picture, the increased use of coeliac-specific serological tests, routine small-bowel biopsy during endoscopy and the general acceptance of the ESPGAN criteria for coeliac disease (Logan et al. 1983, Murray et al. 2003, Collin et al. 2007).

Data concerning the prevalence of dermatitis herpetiformis, so-called “skin coeliac disease”, are scanty. According to Finnish studies the prevalence seems to be close to 0.1% (Collin et al. 1997a, Collin et al. 2007). At the same time as there has been a steady rise in new cases of coeliac disease over two decades, the number of dermatitis herpetiformis cases has remained somewhat stable (Collin et al. 1997a, Collin et al. 2007).

### 2.6.2 Undetected coeliac disease

Since the early 1990s screening studies have uncovered a large number of previously undetected coeliac disease cases. These unrecognized subjects may outnumber those with diagnosed coeliac disease by as much as 5-10:1 (Catassi et al. 1994, Csizmadia et al. 1999).

Scientific questions regarding the clinical significance of undiagnosed coeliac disease and the need for earlier diagnosis even by population mass-screening have been raised. At the moment we know that a major part of the screen-detected cases suffer from different intestinal and extraintestinal symptoms (Johnston et al. 1998, Mäki et al. 2003, West et al. 2003, Bingley et al. 2004, Korponay-Szabo et al. 2007), and decreased bone mineral density has also been evident in undetected disease (Corazza et al. 1996, Mustalahti et al. 1999, West et al. 2003). An association with decreased fertility and increased risk of unfavourable outcome of pregnancy has also been suggested (Sher and Mayberry 1996, Greco et al. 2004).
On the other hand, the cardiovascular risk profile in undetected cases seems to be good as the subjects smoke less, are lighter, have lower cholesterol levels and also possibly lower blood pressure (West et al. 2003). However, before any decision on population-screening programmes, the prognosis of undetected coeliac disease in terms of mortality, and risk of malignancies and fractures should still be evaluated.

2.7 TREATMENT

2.7.1 Principles of treatment

The current treatment for coeliac disease is a strict life-long gluten-free diet, where cereals from the grass tribe Triticae, i.e. wheat, barley and rye, should be avoided (Dicke 1950, Green and Cellier 2007). Oats belong to a separate grass tribe and are nowadays considered harmless for the majority of coeliac disease patients (Janatuinen et al. 2002, Högberg et al. 2004). In addition, there are other grains which can substitute forbidden cereals and sources of starch, for example rice, corn, buckwheat, millet, sorghum, teff, amaranth and quinoa.

As wheat is a staple food for most populations in the world, implementation of a strict gluten-free diet is not a matter of course and thus, a knowledgeable dietician should be consulted at least at the beginning of treatment, and different patient support organizations are also crucial for many patients (Green and Cellier 2007). Any deficiencies in vitamins and minerals, including folic acid, B12, fat-soluble vitamins, iron and calcium, should also be treated (Green and Cellier 2007).

As to the treatment of dermatitis herpetiformis, a gluten-free diet heals the skin as well as abnormalities of the small-bowel mucosa (Fry 2002). However, peroral dapsone (diaminodiphenylsulfone) is an additional therapy for rash in patients with dermatitis herpetiformis, although it has no influence on intestinal abnormalities. Thus, dapsone is mainly used with a gluten-free diet at the beginning of treatment when the effect of the diet on the rash is lacking (Fry 2002).

In future, other more convenient treatment options besides a gluten-free diet will hopefully emerge. One approach is to develop modified wheat (Vader et al. 2003, Molberg et al. 2005), another is to find appropriate medication (Gass et al. 2007, Xia et al. 2007). Although years of intense study will most probably be needed
before any medication is available in clinical practice, the most attractive alternative at the moment is the use of recombinant enzymes to digest the toxic gliadin fractions in the stomach or the upper small intestine (Pyle et al. 2005, Gass et al. 2007).

2.7.2 Response to a gluten-free diet and refractory coeliac disease (sprue)

Rapid amelioration of gastrointestinal symptoms within days or weeks on a gluten-free diet is usual (Pink and Creamer 1967) and a better quality of life as a consequence of treatment in both clinically and screen-detected cases has also been shown (Viljamaa et al. 2005b). As to the complications of coeliac disease, a gluten-free diet seems to improve bone mineral density independent of symptoms (Mustalahti et al. 1999). Dieting also has a potentially beneficial role in fertility problems (Farthing et al. 1982, Tata et al. 2005), neurological illnesses (Cicarelli et al. 2003, Bushara 2005) and in endocrinological conditions (Collin et al. 2002a) and malignancies (Holmes et al. 1989).

Coeliac autoantibodies are usually undetectable within a year on a strict gluten-free diet (Sategna-Guidetti et al. 1993, Dipper et al. 2009). Positive values in treated cases seems to betray non-adherence, but normal values are nevertheless not reliable markers of strict adherence to a gluten-free diet (Troncone et al. 1995, Kaukinen et al. 2002a). Thus, the expertise of a dietician is needed and a small bowel biopsy is still often necessary to confirm the healing of the mucosa. Recovery of histological changes may take months or even years and remain incomplete, especially in adults (Wahab et al. 2002).

The core problem here is that not all patients respond histologically to a gluten-free diet (Wahab et al. 2002, Kaukinen et al. 2007b). The main reason for non-response is intentional or inadvertent gluten ingestion and thus, the first step is to assess adherence to a strict gluten-free diet (Kaukinen et al. 2007b, Malamut et al. 2009). The second step is to review the earlier biopsy, and if necessary, carry out specific investigations keeping differential diagnostic alternatives in mind. After careful examination and implementation a strict gluten-free diet, refractory coeliac disease is a rarity (Kaukinen et al. 2007b, Malamut et al. 2009). Approximately one case of refractory coeliac disease per year was found in each big hospital in France.
between 1992 and 2007 (Malamut et al. 2009). Continuous symptoms and a risk of ulcerative duodenojenunitis as well as malignancies make the refractory disease a serious entity (Malamut et al. 2009). In the treatment of the disease corticosteroids and immunosuppressants such as azathioprine are used (Malamut et al. 2009). The disease entity has been subdivided into two subtypes, I and II, according to a normal or abnormal phenotype of IELs, respectively (Malamut et al. 2009). Type II is characterized by the lack of surface expression of CD3 and CD8 T-cell receptor complexes and a clonal rearrangement of the gamma chain of the T-cell receptor (Malamut et al. 2009). Patients with abnormal IELs are at increased risk of lymphoma, as a major part of them progress into overt lymphoma under surveillance (Al-Toma et al. 2007, Malamut et al. 2009). The 5-year survival rate of these cases seems to be approximately 50% compared to refractory disease type I with a survival rate over 90% (Al-Toma et al. 2007, Malamut et al. 2009).

2.8 MALIGNANCIES

2.8.1 Overall risk of malignancies

The development of malignancy is the most serious complication to affect patients with coeliac disease. Cases with either coeliac disease or dermatitis herpetiformis carry a two- to six-fold increased risk of malignancy at any site according to the studies published before the 1990s (Table 7). However, an increased risk of cancer overall has only been shown in a minority of recent studies, where the risk level has been at maximum 1.4-fold (Table 7). The same phenomenon, a declining risk of malignancies at any site over time, has also been shown in a single cohort study by Askling and colleagues (2002). In evaluation of the studies in question it is necessary to note any methodological issues possibly inducing biased results, for example inclusion of coeliac disease cases without histological confirmation, malignancies prior to coeliac disease diagnosis as well as initiation of follow-up immediately after diagnosis (Table 7).
Table 7. Association of diagnosed coeliac disease (CD) or dermatitis herpetiformis (DH) with malignancies at any site.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Source of participants</th>
<th>Age group</th>
<th>Number of subjects</th>
<th>Mean follow-up time, years</th>
<th>Person years</th>
<th>Risk estimate (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coeliac disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harris et al. 1967</td>
<td>UK</td>
<td>Hospital records</td>
<td>mixed</td>
<td>202</td>
<td>8</td>
<td>ND</td>
<td>4.4 (ND) 2.4</td>
</tr>
<tr>
<td>Selby and Gallagher 1979</td>
<td>Australia</td>
<td>Hospital records</td>
<td>adults</td>
<td>93</td>
<td>ND</td>
<td>ND</td>
<td>5.5 (ND) 4</td>
</tr>
<tr>
<td>Holmes et al. 1989</td>
<td>UK</td>
<td>Hospital records</td>
<td>mixed</td>
<td>210</td>
<td>18</td>
<td>ND</td>
<td>2.0 (1.4-2.8) 5</td>
</tr>
<tr>
<td>Collin et al. 1994</td>
<td>Finland</td>
<td>Hospital records</td>
<td>adults</td>
<td>335</td>
<td>5</td>
<td>ND</td>
<td>1.5 (0.7-2.8)</td>
</tr>
<tr>
<td>Askling et al. 2002</td>
<td>Sweden</td>
<td>Hospital in-patient records</td>
<td>mixed</td>
<td>11,019</td>
<td>ND</td>
<td>97,236</td>
<td>1.3 (1.2-1.5) 5</td>
</tr>
<tr>
<td>Green et al. 2003</td>
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<td>Hospital records</td>
<td>adults</td>
<td>381</td>
<td>6</td>
<td>1,977</td>
<td>1.5 (0.3-7.5) 6</td>
</tr>
<tr>
<td>Card et al. 2004</td>
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<td>5,684</td>
<td>1.0 (0.7-1.5) 5</td>
</tr>
<tr>
<td>West et al. 2004a</td>
<td>UK</td>
<td>General practice research database</td>
<td>mixed</td>
<td>4,732</td>
<td>ND</td>
<td>18,923</td>
<td>1.1 (0.9-1.4) 5</td>
</tr>
<tr>
<td>Viljamaa et al. 2006</td>
<td>Finland</td>
<td>Hospital records</td>
<td>mixed</td>
<td>781</td>
<td>ND</td>
<td>10,956</td>
<td>1.2 (0.9-1.5)</td>
</tr>
<tr>
<td>Anderson et al. 2007</td>
<td>UK</td>
<td>Serological dataset, EMA-positive patients</td>
<td>mixed</td>
<td>490</td>
<td>ND</td>
<td>ND</td>
<td>0.7 (0.4-1.1) 5</td>
</tr>
<tr>
<td>Goldacre et al. 2008</td>
<td>UK</td>
<td>Hospital records</td>
<td>mixed</td>
<td>1,997</td>
<td>11</td>
<td>ND</td>
<td>1.2 (0.9-1.4) 5</td>
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<tr>
<td><strong>Dermatitis herpetiformis</strong></td>
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<td>Leonard et al. 1983</td>
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<td>Hospital records</td>
<td>ND</td>
<td>109</td>
<td>ND</td>
<td>671</td>
<td>2.4 (1.2-3.6)</td>
</tr>
<tr>
<td>Swerdlow et al. 1993</td>
<td>UK</td>
<td>Hospital records</td>
<td>mixed</td>
<td>152</td>
<td>15</td>
<td>2,288</td>
<td>3.9 (1.8-7.5)</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Data source</td>
<td>Type</td>
<td>N</td>
<td>Follow-up</td>
<td>Cases</td>
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<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td>Sigurgeirsson et al. 1994</td>
<td>Sweden</td>
<td>Hospital in-patient records</td>
<td>mixed</td>
<td>976</td>
<td>9</td>
<td>8,662</td>
<td>1.4 (1.1-1.7)</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Collin et al. 1996</td>
<td>Finland</td>
<td>Hospital records</td>
<td>adults</td>
<td>305</td>
<td>10</td>
<td>3,029</td>
<td>1.3 (0.7-2.1)</td>
</tr>
<tr>
<td>Askling et al. 2002</td>
<td>Sweden</td>
<td>Hospital in-patient records</td>
<td>mixed</td>
<td>1,354</td>
<td>ND</td>
<td>14,451</td>
<td>1.2 (1.0-1.4)</td>
</tr>
<tr>
<td>Viljamaa et al. 2006</td>
<td>Finland</td>
<td>Hospital records</td>
<td>mixed</td>
<td>366</td>
<td>ND</td>
<td>6,289</td>
<td>1.0 (0.6-1.5)</td>
</tr>
<tr>
<td>Lewis et al. 2008</td>
<td>UK</td>
<td>General practice research database</td>
<td>mixed</td>
<td>846</td>
<td>4</td>
<td>3,496</td>
<td>1.0 (0.7-1.5)</td>
</tr>
</tbody>
</table>

CI=confidence interval, UK=United Kingdom, ND=not defined, USA=United States of America, EMA=immunoglobulin A class endomysial antibodies
1 Both cases with coeliac disease and idiopathic steatorrhoea without histological confirmation of coeliac disease were included
2 Males
3 Females
4 CI not reported, P-value <0.05
5 Cases with any malignancy within the first 1 or 2 years after the diagnosis of coeliac disease have been excluded
6 Cancers before, simultaneously with and after the diagnosis of coeliac disease were taken into account
7 It is not reported whether EMA-positive cases had received a diagnosis of coeliac disease
Published studies have been limited to patients who have received a clinical diagnosis. As a major part of coeliac disease cases remain unrecognized (Catassi et al. 1994, Csizmadia et al. 1999) the approach in the previous studies has ultimately led to selection of patients. Even though an excess liability to malignancy in undetected cases was suggested decades ago (Stokes et al. 1976), there are still no studies evaluating the overall risk of malignancies in undetected coeliac disease.

2.8.2 Lymphomas

Over 70 years ago subjects suffering from steatorrhoea and associated lymphoma first came to notice and decades later it was suggested that lymphoma is a complication of coeliac disease (Gough et al. 1962). Since then the association with lymphoma and especially non-Hodgkin lymphoma (NHL) has been repeatedly shown (Table 8). In the earliest small studies the association was strong, up to 100-fold, while in recent larger studies the risk estimates have been much lower, mainly between three and six (Table 8). A decreasing risk of NHL over calendar periods has also been shown in a single study by Gao and colleagues (2009); individuals diagnosed in recent years have been estimated to have only a four-fold risk of NHL compared to over 13-fold in cases diagnosed roughly two decades earlier (Gao et al. 2009). In addition, the risk of NHL seems to be greatest within the first years after diagnosis, but to remain high after 10 years´ latency (Cooper et al. 1982, Gao et al. 2009). Higher age at diagnosis of coeliac disease has been suggested to increase the risk of lymphoma, but results are contradictory (Cooper et al. 1982, Freeman 2004, Gao et al. 2009).

The most frequent lymphoma subtype associated with coeliac disease is a high-grade, T-cell NHL of the upper small intestine, named enteropahty-associated T-cell lymphoma (Gough et al. 1962, Verbeek et al. 2008). However, it is a rare malignancy, its incidence in the general population being estimated to be roughly 1 per 1000,000 person years (Lang-Muritano et al. 2002, Verbeek et al. 2008). It occurs in adults, with the incidence peaking in the elderly, and is usually at an advanced stage at diagnosis and has a poor prognosis (Verbeek et al. 2008, Verbeek et al. 2008). Clonal T-cell rearrangements detected in refractory coeliac disease have
been suggested to be a first step in malignant transformation leading to enteropahty-associated T-cell lymphoma (Cellier et al. 2000, Malamut et al. 2009).

The association between coeliac disease and malignant lymphomas is not confined to enteropahty-associated T-cell lymphoma but includes other types of T cell and also B cell NHLs (Catassi et al. 2002, Smedby et al. 2005). The relative risk of T cell NHL seems substantially to outweigh that of B cell NHL, the risk estimates in coeliac disease being reported to be 51- and two-fold, respectively (Smedby et al. 2005). Over and above the 24-fold increased risk of intestinal NHL in coeliac disease, a four-fold increased risk of extraintestinal NHL has also been reported (Smedby et al. 2005).

Whether unrecognized coeliac disease cases carry an increased risk of lymphoma is unclear. Pathological changes in small-bowel biopsy samples typical for coeliac disease were been found in 19% of cases with intestinal lymphoma (Johnston and Watson 2000) In contrast, no increased risk of lymphoma could be detected in a subgroup of unrecognized coeliac disease cases according to a multi-centre case-control study on coeliac disease and NHL (Mearin et al. 2006). In other corresponding case-control studies either unrecognized cases were not searched or the number of such cases was far too low to draw conclusions (Catassi et al. 2002, Farre et al. 2004, Smedby et al. 2006, Gao et al. 2009).
Table 8. Association of coeliac disease (CD) or dermatitis herpetiformis (DH) with non-Hodgkin lymphoma (NHL).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Place of study</th>
<th>Source of participants</th>
<th>Age group</th>
<th>Number of subjects</th>
<th>Mean follow-up time, years</th>
<th>Person years</th>
<th>Risk estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coeliac disease</strong></td>
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<td></td>
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</tr>
<tr>
<td><strong>Cohort studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selby and Gallagher 1979</td>
<td>Australia</td>
<td>CD patients from hospital records</td>
<td>adults</td>
<td>93</td>
<td>ND</td>
<td>ND</td>
<td>RR 49.0 (ND)¹</td>
</tr>
<tr>
<td>Holmes et al. 1989</td>
<td>UK</td>
<td>CD patients from hospital records</td>
<td>mixed</td>
<td>210</td>
<td>18</td>
<td>ND</td>
<td>RR 43.0 (ND)¹</td>
</tr>
<tr>
<td>Askling et al. 2002</td>
<td>Sweden</td>
<td>CD patients from hospital in-patient records</td>
<td>mixed</td>
<td>11,019</td>
<td>97,236</td>
<td>ND</td>
<td>RR 6.3 (4.2-12.5)</td>
</tr>
<tr>
<td>Green et al. 2003²</td>
<td>USA</td>
<td>CD patients from hospital records</td>
<td>adults</td>
<td>381</td>
<td>6</td>
<td>1,977</td>
<td>RR 9.1 (4.7-13.0)</td>
</tr>
<tr>
<td>West et al. 2004a³</td>
<td>UK</td>
<td>CD patients from general practice research database</td>
<td>mixed</td>
<td>4,732</td>
<td>ND</td>
<td>18,923</td>
<td>HR 4.8 (2.7-8.5)</td>
</tr>
<tr>
<td>Card et al. 2004</td>
<td>UK</td>
<td>CD patients from hospital records</td>
<td>mixed</td>
<td>637</td>
<td>7</td>
<td>5,684</td>
<td>RR 5.8 (1.6-14.9)</td>
</tr>
<tr>
<td>Smedby et al. 2005</td>
<td>Sweden</td>
<td>CD patients from hospital in-patient records</td>
<td>mixed</td>
<td>11,650</td>
<td>ND</td>
<td>ND</td>
<td>RR 6.6 (5.0-8.6)</td>
</tr>
<tr>
<td>Viljamaa et al. 2006</td>
<td>Finland</td>
<td>CD patients from hospital records</td>
<td>mixed</td>
<td>781</td>
<td>ND</td>
<td>10,956</td>
<td>RR 3.2 (1.0-7.5)</td>
</tr>
<tr>
<td>Silano et al. 2007</td>
<td>Italy</td>
<td>CD patients from hospital records</td>
<td>mixed</td>
<td>1,968</td>
<td>ND</td>
<td>ND</td>
<td>RR 4.7 (2.9-7.3)</td>
</tr>
<tr>
<td>Goldacre et al. 2008</td>
<td>UK</td>
<td>CD patients from hospital records</td>
<td>mixed</td>
<td>1,997</td>
<td>11</td>
<td>ND</td>
<td>RR 3.3 (1.5-6.3)</td>
</tr>
<tr>
<td><strong>Case-control studies</strong></td>
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</tr>
<tr>
<td>Catassi et al. 2002</td>
<td>Italy</td>
<td>NHL patients from diagnostic centres</td>
<td>adults</td>
<td>653</td>
<td>ND</td>
<td>ND</td>
<td>OR 3.1 (1.3-7.6)</td>
</tr>
<tr>
<td>Farre et al. 2004³</td>
<td>Spain</td>
<td>NHL patients from diagnostic centres</td>
<td>adults</td>
<td>298</td>
<td>ND</td>
<td>ND</td>
<td>OR 0.6 (0.1-3.8)</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Sample Description</td>
<td>Group</td>
<td>N</td>
<td>CI</td>
<td>RR</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
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</tr>
<tr>
<td>Mearin et al. 2006</td>
<td>Europe</td>
<td>NHL patients from diagnostic centres</td>
<td>adults</td>
<td>1,446</td>
<td>ND</td>
<td>ND</td>
<td>OR 2.6 (1.4-4.9)</td>
</tr>
<tr>
<td>Smedby et al. 2006</td>
<td>Sweden</td>
<td>NHL patients from Swedish and Danish cancer registries</td>
<td>adults</td>
<td>3,055</td>
<td>ND</td>
<td>ND</td>
<td>OR 2.1 (1.0-4.8)</td>
</tr>
<tr>
<td>Gao et al. 2009</td>
<td>Sweden</td>
<td>NHL patients from the Swedish cancer registry</td>
<td>adults</td>
<td>37,869</td>
<td>ND</td>
<td>ND</td>
<td>OR 5.4 (3.6-8.1)</td>
</tr>
</tbody>
</table>

**Dermatitis herpetiformis**

*Cohort studies*

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Sample Description</th>
<th>Group</th>
<th>N</th>
<th>CI</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leonard et al. 1983</td>
<td>UK</td>
<td>DH patients from hospital records</td>
<td>ND</td>
<td>109</td>
<td>ND</td>
<td>671</td>
</tr>
<tr>
<td>Sigurgeirsson et al. 1994</td>
<td>Sweden</td>
<td>DH patients from hospital in-patient records</td>
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<td>8,662</td>
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<td>DH patients from hospital records</td>
<td>adults</td>
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<td>10</td>
<td>3,029</td>
</tr>
<tr>
<td>Askling et al. 2002</td>
<td>Sweden</td>
<td>DH patients from hospital in-patient records</td>
<td>mixed</td>
<td>1,354</td>
<td>ND</td>
<td>14,451</td>
</tr>
<tr>
<td>Viljamaa et al. 2006</td>
<td>Finland</td>
<td>DH patients from hospital records</td>
<td>mixed</td>
<td>366</td>
<td>ND</td>
<td>6,289</td>
</tr>
<tr>
<td>Lewis et al. 2008</td>
<td>UK</td>
<td>DH patients from the general practice research database</td>
<td>mixed</td>
<td>846</td>
<td>4</td>
<td>3,496</td>
</tr>
</tbody>
</table>

CI=confidence interval, RR=relative risk, ND=not defined, UK=United Kingdom, USA=United States of America, OR=odds ratio
1 CI not reported, P-value < 0.001
2 Non-Hodgkin lymphomas before, simultaneously and after the diagnosis of coeliac disease were taken into account
3 Overall risk of lymphoma was evaluated
4 Males
5 Females
2.8.3 Other malignancies

An increase in gastrointestinal carcinomas in diagnosed coeliac disease cases was reported over 40 years ago (Harris et al. 1967), and while the association has not been shown in all pertinent studies (Card et al. 2004, West et al. 2004a, Viljamaa et al. 2006), several specific malignancies of the gastrointestinal tract have repeatedly been evidenced as being associated with coeliac disease. These include oropharyngeal and oesophageal malignancies (Holmes et al. 1976, Swinson et al. 1983, Holmes et al. 1989, Askling et al. 2002, Green et al. 2003) and adenocarcinoma of the small intestine (Askling et al. 2002, Green et al. 2003, Silano et al. 2007). Malignancies of the large intestine and liver and melanoma of the skin have also been suggested to be associated with coeliac disease, but results have been inconclusive (Askling et al. 2002, Green et al. 2003). On the other hand, the risk of breast cancer has been seen to be decreased among diagnosed coeliac disease patients in some study settings (Askling et al. 2002, Silano et al. 2007).

2.8.4 Effect of a gluten-free diet on malignancies

Evidence of the effect of a gluten-free diet on the risk of malignancies is mainly indirect and sparse. The overall risk of malignancy and the risk of lymphoma have been shown to be greatest within the first years after diagnosis and to decline thereafter (Cooper et al. 1982, Askling et al. 2002, Card et al. 2004, Silano et al. 2008, Gao et al. 2009). High age at diagnosis of coeliac disease has also been held to expose individuals to lymphoma and malignancies at any site, but results have been contradictory (Cooper et al. 1982, Freeman 2004, Silano et al. 2007, Gao et al. 2009). In addition, the majority of patients with coeliac disease or dermatitis herpetiformis complicated by lymphoma have not kept a strict gluten-free diet (Leonard et al. 1983, Hervonen et al. 2005, Viljamaa et al. 2006, Silano et al. 2008).

In the well-known study by Holmes and colleagues (1989) the overall risk of malignancies was not statistically significantly increased in individuals adhering strictly to a gluten-free diet over five years. In contrast, subjects taking a normal or a reduced gluten diet had 2.6-fold cancer morbidity compared to the general population. Apart from age and sex no other confounding factors were taken into
account in the study and further, malignancies soon after diagnosis of coeliac disease were not excluded. According to the same study, the risk estimates for carcinoma of the mouth, pharynx and oesophagus as well as NHL were substantially lower in the strictly treated group compared to the group with normal or reduced gluten diets. This has to be interpreted with caution, as the numbers of cases with the defined malignancies were low. Strict adherence to a gluten-free diet was also thought to explain the good prognosis of Finnish individuals in respect of the overall risk of malignancies (Collin et al. 1994). In addition, the risk of enteropahty-associated T-cell lymphoma seems to depend on the presence of gluten in the diet (Silano et al. 2008). However, there is so far no evidence as to whether imposing a gluten-free diet on individuals found by screening prevents malignant complications.

2.9 MORTALITY

2.9.1 Overall risk of mortality

Studies with diagnosed coeliac disease have shown an increased risk of overall mortality with only one exception (Table 9). In these studies, carried out in Scandinavia, the United Kingdom and Italy, risk estimates have varied between 1.3 and 3.8. The lowest risk estimate has been reported in the largest study so far. Interestingly, the risk of mortality in patients with dermatitis herpetiformis has not been increased but rather decreased (Table 9).

Table 9. Association of diagnosed coeliac disease (CD) and dermatitis herpetiformis (DH) with overall mortality.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Place of study</th>
<th>Source of participants</th>
<th>Age group</th>
<th>Number of subjects</th>
<th>Mean follow-up time, years</th>
<th>Person years</th>
<th>Risk estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coeliac disease</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nielsen et al. 1985</td>
<td>Denmark</td>
<td>Hospital records</td>
<td>mixed</td>
<td>98</td>
<td>ND</td>
<td>ND</td>
<td>3.4 (ND) ^2</td>
</tr>
<tr>
<td>Logan et al. 1989</td>
<td>UK</td>
<td>Several sources</td>
<td>mixed</td>
<td>653</td>
<td>14</td>
<td>8,823</td>
<td>1.9 (1.5-2.2)</td>
</tr>
<tr>
<td>Collin et al. 1994</td>
<td>Finland</td>
<td>Hospital records</td>
<td>adults</td>
<td>335</td>
<td>5</td>
<td>ND</td>
<td>ND ^4</td>
</tr>
<tr>
<td>Cottone et al. 1999</td>
<td>Italy</td>
<td>Hospital records</td>
<td>adults</td>
<td>216</td>
<td>6</td>
<td>ND</td>
<td>3.8 (2.0-7.0)</td>
</tr>
<tr>
<td>Corrao et al. 2001</td>
<td>Italy</td>
<td>Hospital records</td>
<td>adults</td>
<td>1,072</td>
<td>6</td>
<td>6,444</td>
<td>2.0 (1.5-2.7)</td>
</tr>
<tr>
<td>Peters et al. 2003</td>
<td>Sweden</td>
<td>Hospital in-patient records</td>
<td>mixed</td>
<td>10,032</td>
<td>8</td>
<td>81,182</td>
<td>2.0 (1.8-2.1) ^5</td>
</tr>
<tr>
<td>West et al. 2004a</td>
<td>UK</td>
<td>General practice research database</td>
<td>mixed</td>
<td>4,732</td>
<td>ND</td>
<td>18,923</td>
<td>1.4 (1.2-1.6)</td>
</tr>
<tr>
<td>Viljamaa et al. 2006</td>
<td>Finland</td>
<td>Hospital records</td>
<td>mixed</td>
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<td>17,245</td>
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<tr>
<td>Anderson et al. 2007</td>
<td>UK</td>
<td>Serological dataset, EMA-positive patients suspected to have CD ^6</td>
<td>mixed</td>
<td>490</td>
<td>ND</td>
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<td>1.8 (1.3-2.3)</td>
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<tr>
<td>Solaymani-Dodaran et al. 2007</td>
<td>UK</td>
<td>Several separate sources ^3</td>
<td>children</td>
<td>285</td>
<td>34</td>
<td>14,926</td>
<td>2.6 (1.6-4.0)</td>
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<tr>
<td>Ludvigsson et al. 2009</td>
<td>Sweden</td>
<td>Nationwide pathology data</td>
<td>mixed</td>
<td>29,096</td>
<td>ND</td>
<td>ND</td>
<td>1.3 (1.2-1.3)</td>
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</table>
### Dermatitis herpetiformis

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Data Source</th>
<th>Method</th>
<th>Total</th>
<th>Cases</th>
<th>Follow-Up</th>
<th>RR</th>
<th>CI</th>
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</thead>
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<tr>
<td>Swerdlow et al. 1993</td>
<td>UK</td>
<td>Hospital records</td>
<td>mixed</td>
<td>152</td>
<td>15</td>
<td>2,288</td>
<td>0.9</td>
<td>(0.6-1.2)</td>
</tr>
<tr>
<td>Collin et al. 1996</td>
<td>Finland</td>
<td>Hospital records</td>
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<td>305</td>
<td>10</td>
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<td>ND</td>
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<td>Viljamaa et al. 2006</td>
<td>Finland</td>
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<td>mixed</td>
<td>366</td>
<td>ND</td>
<td>6,289</td>
<td>0.5</td>
<td>(0.4-0.7)</td>
</tr>
<tr>
<td>Lewis et al. 2008</td>
<td>UK</td>
<td>General practice research database</td>
<td>mixed</td>
<td>846</td>
<td>4</td>
<td>3,496</td>
<td>0.9</td>
<td>(0.7-1.2)</td>
</tr>
</tbody>
</table>

Cl=confidence interval, ND=not defined, UK=United Kingdom, EMA=immunoglobulin A-class endomysial antibodies

1 24% of the participants did not respond to a gluten-free diet
2 Cl not reported, P<0.025
3 Hospital records, in-patient statistics and histopathology records, the Coeliac Society, postal survey for general practitioners
4 The difference from comparison group was not statistically significant
5 The first year of follow-up was excluded from the analysis
6 It is not reported whether EMA-positive cases had received a diagnosis of coeliac disease
2.9.2 Effect of a gluten-free diet on mortality

There are only few studies evaluating the effect of a gluten-free diet on mortality. The risk of mortality seems to be highest soon after diagnosis and to decline gradually thereafter, which might indirectly bespeak a beneficial effect of the diet (Logan et al. 1989, Cottone et al. 1999, Corrao et al. 2001, West et al. 2004a, Viljamaa et al. 2006, Solaymani-Dodaran et al. 2007, Ludvigsson et al. 2009). On the other hand, a high risk of mortality soon after the diagnosis of coeliac disease may merely reflect ascertainment bias. In a study by Corrao and associates (2001), coeliac disease patients were stratified according to the duration of delay in diagnosis as well as to adherence to a gluten-free diet. The overall risk of mortality among coeliac disease patients was found to be two-fold, while in subjects with the diagnostic delay more than ten years the risk was 3.8-fold. The observation suggests that long duration of untreated illness has a disadvantageous effect independent of forthcoming diet. However, direct evidence of a beneficial effect of a gluten-free diet on the overall risk of mortality has also been reported, as non-adherent patients had a six-fold increased risk of mortality compared to those adhering (Corrao et al. 2001). Evidence is lacking as to whether a gluten-free diet could also have a role in reducing the potentially increased risk of overall mortality in undetected condition.

2.9.3 Cause-specific mortality

Malignant diseases have also been overrepresented as a cause of death in diagnosed coeliac disease, with increased risks between 1.6 and 3.6 (Logan et al. 1989, Corrao et al. 2001, Peters et al. 2003, Viljamaa et al. 2006, Solaymani-Dodaran et al. 2007, Ludvigsson et al. 2009). The malignancies in question included lymphomas and malignancies of the gastrointestinal tract. The association with non-malignant digestive system diseases as cause of death would also appear obvious (Corrao et al. 2001, Peters et al. 2003, Viljamaa et al. 2006). And increased risk of circulatory system diseases in diagnosed cases has been reported (Ludvigsson et al. 2007a, Wei et al. 2008). However, an increased risk of cardiovascular diseases as cause of death
was reported only in the largest study so far (Logan et al. 1989, Corrao et al. 2001, Viljamaa et al. 2006, Anderson et al. 2007, Ludvigsson et al. 2009).

2.9.4 Mortality in undetected coeliac disease

There are only few studies addressing the effect of unrecognized coeliac disease on mortality. Johnston and colleagues (1998) found 8.5% of 1204 subjects from the general population to carry either IgA-class antigliadin, antireticulin or antiendomysial autoantibodies. Mortality was compared between the antibody-positive individuals and the general population in a follow-up of 12 years and a standardized mortality ratio (SMR) was defined (0.9, 95% CI 0.5-1.6). However, due to the previously reported low specificity of AGA (Rostom et al. 2005), several antibody-positive subjects most probably had no coeliac disease, this possibly weakening the real association. Correspondingly, West and colleagues (2003) found no difference in the proportion of deaths recorded in EMA-positive screen-detected participants from the general population (n=87) compared to EMA-negatives in a surveillance of 6 to 11 years. However, the analysis was of limited power, as only five deaths were reported in surveillance. In contrast, Metzger and associates compared screen-detected tTG-ab-positive individuals from the general population (n=63) to tTG-ab-negative participants in respect of mortality over a period of eight years and found a 2.5–fold increased risk (95% CI 1.5-4.3). The excess of cancer mortality in tTG-ab-positive cases was even higher, 3.6-fold (95% CI 1.7-7.8). In conclusion, it remains unclear whether most subjects with coeliac disease who remain undiagnosed and thus untreated have a normal or reduced life expectancy.
3. PURPOSE OF THE STUDY

The main aims of the present study were to describe changes in the total prevalence of coeliac disease in Finland at time-points 20 years apart and to evaluate the prognosis of undetected coeliac disease in terms of malignancies and mortality. The specific objectives were:

1. To assess the prevalence of diagnosed and undetected coeliac disease as well as the total prevalence of the disease in Finnish adults over 30 years of age in 1978-80 and 2000-01 and to ascertain whether any changes have taken place in the figures over time (I).

2. To establish whether Finnish adults over 30 years of age with unrecognized coeliac disease carry an increased risk of overall malignancy or any site-specific malignancies (II).

3. To establish whether undetected coeliac disease in Finns over 30 years of age is associated with all-cause or cause-specific mortality (III).
4. PARTICIPANTS AND METHODS

4.1 Mini-Finland survey (I-III)

An extensive epidemiological study of the adult Finnish population over 30 years of age, the Mini-Finland survey (I-III), was carried out in 1978-80 (Aromaa et al. 1989). The primary aim of the study, conducted by the Social Insurance Institution of Finland, was to obtain a comprehensive picture of Finnish adults’ health, functional capacity and need of care. The sampling method used was a two-stage stratified cluster design planned by Statistics Finland (Aromaa et al. 1986, Aromaa et al. 1989). One or more neighbouring municipalities on the mainland were defined to form 320 clusters. The clusters were combined into 40 strata of 40,000-60,000 individuals according to the proportion of the population living from industry and agriculture, as well as to population density. In the first stage of sampling, one cluster from the each stratum and in the second stage, 8,000 individuals from the 40 clusters were selected at random. The survey comprised questionnaires, interviews, clinical examination by a physician and collection of blood samples. Altogether 7,217 persons, i.e. 90% of the whole sample participated (Figure 1 in I) (Aromaa et al. 1989). A total of 6,993 sera were still available for the purposes of the current study in 2001-02 (Figure 1 in I). Age and sex adjusted characteristics of the participants in the Mini-Finland survey are shown in Table 1 in I.

4.2 Health 2000 survey (I)

In the Health 2000 survey, directed by the then National Public Health Institute, 8,028 individuals representing Finnish adults aged 30 and over were sampled in 2000-01 (Figure 1 in I) (Aromaa and Koskinen 2004). One of the goals of the study was to compare population health and functional capacity over time, between the Mini-Finland and the Health 2000 surveys. The sampling method used in 2000-01
was a stratified two-stage cluster sampling design comparable to that used in the Mini-Finland survey (Aromaa and Koskinen 2004). The population on the mainland was divided into five strata according to university hospital districts, each containing roughly one million inhabitants. In the first stage of sampling, 16 health centre districts from each of the five university hospital regions were sampled, yielding altogether 80 out of 249 districts. The 15 largest health centre districts in Finland were selected in the sample with probability 1 and the remaining 65 were chosen at random. In the second stage, 8,028 individuals from the defined areas were selected by systematic sampling. The age group 80 or over was oversampled to make sure that a sufficient number of old examinees were included in the study. The oversampling was taken into account in data analysis. In addition to the drawing of blood samples, the Health 2000 survey comprised questionnaires, interviews and clinical examination principally similar to those employed in the Mini-Finland survey. A total of 6,770 individuals participated, yielding a participation rate 84% in the primary study (Figure 1 in I) (Aromaa and Koskinen 2004). There were still 6,402 sera available for the current study in 2001-02 (Figure 1 in I). Age and sex adjusted characteristics of the participants in the Health 2000 survey are shown in Table 1 in I.

### 4.3 Cases with previously diagnosed coeliac disease (I-III)

In both surveys participants were asked whether they had any chronic diseases and in the case of a positive answer, the specific illness was asked (I-III) (Aromaa et al. 1989, Aromaa and Koskinen 2004). Chronic conditions were also observed in the course of clinical examinations (I-III). In addition, the participants in the Health 2000 survey were asked separately, whether a physician had previously diagnosed coeliac disease (I). In 2004, the reported diagnoses of coeliac disease or dermatitis herpetiformis in the Health 2000 cohort were scrutinized by case record data (I) and diagnoses in the Mini-Finland survey by medical certificates basing on case record data (I-III). Only coeliac disease and dermatitis herpetiformis cases fulfilling the diagnostic criteria for the diseases were included in (Figure 1 in I) or excluded from analysis (Figure 1 in II-III). The diagnosis of coeliac disease was based on both
villous atrophy with crypt hyperplasia and clinical or histological recovery on a gluten-free diet (Walker-Smith et al. 1990). Before the 1970s the diagnosis of dermatitis herpetiformis was based on typical clinical picture and thereafter on the demonstration of pathognomic granular IgA deposits in the dermal papillae by direct immunofluorescence examination (Reunala et al. 1984, Fry 2002).

4.4 Cases with undetected coeliac disease (I-III)

The previously collected blood samples were stored at -20°C until 2001-02, when undetected coeliac disease was defined by a two-stage serological screening algorithm (I-III). A total of 6,993 sera from participants in the Mini-Finland survey (3,771 females) and 6,402 from the Health 2000 survey (3,527) were still available for the purposes of the present study (I-III, Figure 1 in I). The age and sex distributions of the participants with available sera are shown in Table 2 in I. All available sera were tested for tTG-ab (Eu-tTG® umana IgA, Eurospital S.p.A, Trieste, Italy, abbreviated as Eu-tTG in the present study) and positive sera were further analysed for EMA (I-III) and, tTG-ab by another kit (Celikey®, Phadia, Freiburg, Germany, abbreviated as Celikey tTG) (II-III), see figure 1 in I-III. Both commercial tissue transglutaminase antibody kits use human recombinant tissue transglutaminase as antigen and results are given in arbitrary units (AU). The cut-off point for the Eu-tTG was 7.0 AU/ml and for Celikey tTG 5.0 AU/ml according to manufacturers’ instructions. In the definition of EMA, a standardized and validated indirect immunofluorescence method using human umbilical cord as antigen was used (Ladinser et al. 1994, Sulkanen et al. 1998, Stern and Working Group on Serologic Screening for Celiac Disease 2000). The test result was considered positive when a characteristic staining pattern at a serum dilution 1:≤5 was detected. The definition of undetected coeliac disease was based on the defined two-stage screening algorithm, where cases yielding a positive result for both Eu-tTG and either EMA (I-III) or Celikey tTG (II-III) were considered to have undetected disease.

The current study brought out an unexpectedly high proportion of Eu-tTG positivity (8%) in the sera from the Mini-Finland cohort collected 22 years earlier (Figure 1 in I-III). To confirm that the likelihood of coeliac disease was low in
individuals negative for Eu-tTG, one in 50 (n=128) of the serum samples was further analysed for Celikey tTG and EMA. As none proved positive for either of the antibodies, the influence of storage on Eu-tTG values seemed not to be random, rather induced by a serum concentration effect. Further, the stability of coeliac autoantibodies during the long storage at -20°C was also evaluated by 12 separate sera positive for EMA, an average of 14 (11 to 18) years earlier. The sera drawn from biopsy-proven untreated coeliac disease patients were reanalysed for EMA in the laboratory blinded to the primary results. As the EMA result remained similar in all serum samples, storage can hardly have had a diluting effect on the sera.

4.5 Total prevalence of coeliac disease (I)

To calculate the total prevalence of coeliac disease both cases with previously diagnosed coeliac disease or dermatitis herpetiformis and hitherto unrecognized screen-detected EMA-positive cases were taken into account (I). The number of participants with an available serum sample in 2001-02, i.e. 6,993 in the Mini-Finland and 6,402 in the Health 2000 survey, were used as denominators in calculating the prevalence of coeliac disease. Information on age, sex and education was extracted for adjustment purposes.

4.6 Follow-up of cases with undetected coeliac disease as regards malignancies (II)

In order to evaluate the risk of overall malignancy and site-specific malignancies in undetected coeliac disease the personal identification codes of the participants in the Mini-Finland survey were linked with records from the nationwide database of the Finnish Cancer Registry (II). The cases with a history of any malignancy at the beginning of follow-up (n=141) were excluded from the analysis (Figure 1 in II). The follow-up commenced the day the blood samples were drawn in 1978-80 and the participants were followed up for a maximum of 19 years until the emergence of cancer, death or the end of 1996, whichever came first. Follow-up of cases with undetected coeliac disease in respect of malignancies yielded a total follow-up of
103,815 person years. Malignancies at any site as well as site-specific malignancies were compared between antibody-positive and -negative individuals in the Mini-Finland cohort (II). Site-specific malignancies compared in the present study were the most common cancers in Finland (breast, prostate, lung) as well as malignancies previously shown to be associated with coeliac disease (lymphomas and malignancies of the gastrointestinal tract). Information on age, sex, smoking, body mass index, alcohol consumption, physical activity, consumption of bread, education, number of births and menopausal status were extracted for adjustment purposes (Aromaa et al. 1989).

4.7 Follow-up of cases with undetected coeliac disease as regards mortality (III)

To assess mortality in undetected coeliac disease personal identification codes were linked to the nationwide database of Statistics Finland (III). The date and principal causes of death of the participants in the Mini-Finland survey were extracted from the database. Causes of death were coded either according to the International classifications of diseases (ICD) -8, -9 or -10 depending on the time of death. The study subjects were under surveillance since the blood samples were drawn in 1978-80 until the end of 2005 or death, this yielding the total follow-up of 147,646 person years. The maximum follow-up time was 28 years (mean 20, range 0-28 years). Overall mortality and specific causes of death were compared between antibody-positive and -negative individuals in the Mini-Finland cohort. All main groups of ICD were evaluated as causes of death, as well as malignancies known to be associated with coeliac disease (lymphomas and malignancies of digestive organs) (III). Information on age, sex, education, body mass index, alcohol consumption, smoking, hypertension, serum cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, diabetes, coronary heart disease, stroke and cancer were extracted for purposes of adjustment (Aromaa et al. 1989).
4.8 Statistical analysis (I-III)

To estimate adjusted prevalences with 95% CIs and ORs between the surveys, a logistic regression model was used (I). Age, sex, educational level, smoking and the surveys were included in the models when calculating ORs (I). P-values were calculated using Satterthwaite F-test (I).

To estimate adjusted risks and their 95% CIs for malignancies and mortality, a Cox regression model was used (II-III) (Cox 1972). Statistical heterogeneity was tested using the likelihood ratio test based on the model and expressed by P-value (II-III). P-values ≤0.05 were considered statistically significant (I-III).

The possible confounding effects of age (II-III), sex (II-III), education (II-III), body mass index (II-III), alcohol consumption (II-III), smoking status (II-III), physical activity (II), consumption of bread (II), hypertension (III), serum cholesterol (III), high-density lipoprotein (III), triglycerides (III), diabetes (III), coronary heart disease (III), stroke (III) and cancer (III) on overall risks of malignancies and mortality were assessed using a series of multivariate models. Menopausal status and number of births were also adjusted for in analysis of the association between undetected coeliac disease and breast cancer (II). In a separate analysis of NHL, age, sex and alcohol consumption were adjusted for (II).

Multiplicative interaction terms were used to assess the possible interactions between antibody status and age, sex, smoking and body mass index in the overall risk of malignancies (II). The corresponding factors tested in the model with antibody status in study III were age, sex, smoking, body mass index, alcohol consumption, hypertension, cholesterol levels and education.

Stratified analyses were conducted to assess the overall risk of malignancies according to the level of antibodies and EMA status in tTG-ab-positive individuals (II). To estimate overall risk of malignancies at different levels of antibodies, Celikay tTG-positive cases were divided into tertiles. In addition, the risk was also estimated after exclusion of EMA-positive cases from the tTG-ab-positive individuals (II). In turn, individuals positive for either Celikay tTG or EMA in study III were bisected by medians of positive values (titres 1:<500 and 1: ≥500 in EMA and <6.4 and ≥6.4 AU/ml in Celikay tTG) and overall risk of mortality was evaluated in these subgroups. All-cause mortality was also assessed over the course
of time, the first ten years of follow-up and the surveillance thereafter were analysed separately (III).

The analyses were performed using SAS 8.02 (I-II) (SAS Institute, Cary, North Carolina, United States of America abbreviated as USA) and 9.1 (III) (SAS Institute, Cary, North Carolina, USA) as well as SUDAAN 9.0.0 (I) (Survey Data Analysis, Research Triangle Institute, Research Park Triangle, North Carolina, USA) statistical software, which takes into account sampling weights.

4.9 Ethics (I-III)

Declaration of Helsinki ethical principles on human experimentation were followed. All participants gave informed consent in both surveys and data were analysed anonymously. The ethical committee of Tampere University Hospital approved the current study protocol.
5. RESULTS

5.1 Prevalence of coeliac disease over time

The prevalence of clinically diagnosed coeliac disease increased from 0.03% (n=2, 95% CI 0-0.07%) in 1978-80 to 0.52% (n=32, 95% CI 0.35-0.68%) in 2000-01 (Figure 1). Thus, the age- and sex-adjusted risk of receiving a diagnosis of coeliac disease was roughly 20-fold (OR 25.28, 95% CI 6.12-104.40) in the Health 2000 cohort (2000-01) compared to the earlier Mini-Finland cohort (1978-80).

As to the prevalence of undetected coeliac disease, 577 (8.25%) out of the 6,993 analysed serum samples were positive for Eu-tTG (median value 8.4 AU/ml, lower quartile 7.5, upper quartile 10.0, range 7.1-25.0) in the Mini-Finland survey carried out in 1978-80 (Figure 1 in ). Further, 74 (12.82%) out of 577 Eu-tTG-positive samples also proved positive for EMA, representing unrecognized cases in the present study and yielding a screen-detected prevalence of 1.03% (95% CI 0.79-1.27) (Figure 1). The majority (53 out of 74, 72%) of unrecognized cases were females. Correspondingly, 129 (2.02%) of the 6,402 analysed serum samples from the Health 2000 survey were positive for Eu-tTG (median value 16.2 AU/ml, lower quartile 9.9, upper quartile 21.0, range 7.1-26.0) and the number of unrecognized coeliac disease cases with a positive test result for EMA was 92 (57 females, 62%), yielding a prevalence of 1.47% (95% CI 1.17-1.77) in 2000-01 (Figure 1). The age and sex adjusted risk of having undetected coeliac disease was statistically significantly increased over two decades (OR 1.45, 95% CI 1.06-1.99).

In 1978-80, 97% of all coeliac disease cases remained unrecognized while in 2000-01, 74% of the population with the condition had still not got a proper diagnosis.

Taking into account previously diagnosed (n=2) and unrecognized (n=74) coeliac disease cases, the age and sex adjusted total prevalence of coeliac disease was 1.05% (95% CI 0.80-1.29) in 1978-80 (Figure 1). In 2000-01, the total prevalence was statistically significantly higher, as the sum of 32 diagnosed and 92 screen-
detected cases yielded an age and sex adjusted prevalence of 1.99% (95% CI 1.64-2.33, P=0.004). Thus, the age and sex adjusted risk of having either diagnosed or unrecognized coeliac disease increased nearly two-fold (OR 1.94, 95% CI 1.44-2.60) over the time-span examined. Further adjustment for smoking had no substantial effect on the risk level (OR 2.00, 95% CI 1.49-2.68). The age adjusted total prevalence increased from 0.65% (95% CI 0.41-0.89) to 1.65% (95% CI 1.16-2.14) in men and from 1.40% (95% CI 1.05-1.75) to 2.29% (95% CI 1.78-2.80) in women. An increasing trend in the total prevalence of coeliac disease could also be seen in all age groups, even though the rise was only statistically significant in the age groups 30-44 and 45-54 years (Table 3 in I).

Figure 1. The total prevalence of coeliac disease in 1978-80 and 2000-01 (I). Verticals indicate 95% confidence intervals.

![Graph showing prevalence of coeliac disease]

5.2 Prognosis of undetected coeliac disease as regards malignancies and mortality

After exclusion of clinically diagnosed cases with any malignancy (II) and coeliac disease or dermatitis herpetiformis cases (II-III) at the beginning of the follow-up, altogether 6,849 (II) / 6,987 (III) participants took part in our two-stage serological
screening procedure (Figure 1 in II-III). A total of 565 (II) / 574 (III) analysed sera were positive for Eu-tTG. In study II, a further 202 [129 (64%) females, mean age 59 years] Eu-tTG-positive cases were also Celikey tTG-positive and 73 [52 females (71%), mean age 50 years] EMA-positive (Figure 1 in II). The corresponding figures in study III were 204 participants [125 (61%) females, mean age 59 years] positive for Celikey tTG and 74 [53 (72%) females, mean age 49 years] for EMA. Thus, 1.1% of the samples analyzed were EMA-positive and, correspondingly, 2.9% Celikey tTG-positive (II-III).

As to the personal characteristics of the screened population, subjects positive for either EMA or Celikey tTG were more likely to be women, and had better cholesterol profiles compared to antibody-negative participants (Table 1 in III). Total cholesterol levels (6.36 versus 6.95 mmol/L as regards EMA-positivity, P<0.001; 6.37 versus 6.96 mmol/L as regards Celikey tTG-positivity, P<0.001) as well as LDL (4.24 versus 4.56 mmol/L as regards EMA-positivity, P=0.02; 4.15 versus 4.57 mmol/L as regards Celikey tTG-positivity, P<0.001) and HDL (1.50 versus 1.70 mmol/L as regards EMA-positivity, P<0.001; 1.55 versus 1.70 mmol/L as regards Celikey tTG-positivity, P<0.001) were statistically significantly lower in antibody-positive compared to –negative participants. In addition, triglycerides were also lower in antibody-positive cases (1.38 versus 1.54 mmol/L as regards EMA-positivity; P=0.19; 1.50 versus 1.53 mmol/L as regards Celikey tTG-positivity, P=0.67), but the difference did not reach statistical significance. The subjects positive for Celikey tTG were older (59.1 versus 50.8 years, P<0.001), consumed more alcohol (60.8 versus 45.3 g/wk, P=0.03) and more likely suffered from diabetes (11.1 versus 5.5%, P<0.001) than negative ones (the figures are from Table 1 in III, but the corresponding figures can also be seen in Table 1 in II). Otherwise, no statistically significant differences across antibody status were detected.

Altogether 694 (10.13%) of the participants in study II developed a malignancy of some type by the end of 1996. Persons who developed cancer were older (58.8 versus 49.9 years, P<0.001) and were more likely to be men (51.8 versus 45.8%, P=0.01) or smokers (30.0 versus 23.0%, P<0.001). No association between coeliac autoantibody positivity and malignancy at any site could be detected, as the age and sex adjusted hazard ratio (HR) of overall malignancy was 0.67 (95% CI 0.28-1.61) in EMA-positive cases and correspondingly 0.91 (95% CI 0.60-1.37) in Celikey tTG-positive individuals (Figure 2). Due to the relatively low number of EMA-
positive cases with cancer multivariate adjusting was performed only in Celikey tTG-positive cases, this having no substantial effect on the risk level (HR 0.95, 95% CI 0.62-1.44). Nor was any association found in different levels of Celikey tTG or after exclusion of EMA-positive cases from Celikey tTG-positive individuals (II, data not shown). No statistically significant interactions between any of the potentially effect-modifying factors (age, sex, smoking and body mass index) and antibody status were noted in the prediction of malignancy at any site (II).

The above notwithstanding, the study II uncovered an increased risk of specific malignancies such as NHL and carcinoma of the oesophagus. The age and sex adjusted risk of NHL was 6.43 (95% CI 1.52-27.22, p=0.05, N=2) in EMA-positive cases, while the corresponding risk for Celikey tTG-positive individuals was 2.92 (95% CI 0.87-9.74, p=0.13, N=3). The time-span between known antibody positivity and the diagnosis of NHL varied between 6 and 14 years and there were no enteropathy-associated T-cell lymphomas or any other site predilections (Table 3 in II). Further, the age and sex adjusted risk of carcinoma of the oesophagus was also increased among Celikey tTG-positive cases (HR 7.48, 95% CI 2.06-27.25, p=0.01, N=3). Further adjustment for alcohol consumption did not considerably alter the risk of NHL and carcinoma of the oesophagus in antibody-positive individuals.

Study III revealed that altogether 3,069 (43.9%) of the participants had died by the end of 2005. In parallel with the overall risk of malignancies, no increased risk of mortality could be detected among EMA-positive individuals (age and sex adjusted HR 0.78, 95% CI 0.52-1.18, P= 0.22) (Figure 3). However, Celikey tTG-positive cases carried a border-line significant modestly elevated risk of overall mortality (age and sex adjusted HR 1.19, 95% CI 0.99-1.42, P= 0.07). Multivariate adjustment did not considerably change the figures (Table 2 in III). Individuals with high EMA titres had likewise no increased risk of mortality, but cases with the highest Celikey tTG levels had a border-line significantly elevated risk of overall mortality (HR 1.32, 95% CI 1.00-1.72, P=0.12). The risks over the first 10 years of follow-up [EMA-positive 0.36 (95% CI 0.12-1.11, P=0.03); Celikey tTG-positive 1.17 (95% CI 0.89-1.54, P=0.26)] did not differ statistically significantly from those beyond that period [EMA-positive 0.95 (95% CI 0.61-1.47, P=0.81); Celikey tTG-positive 1.23 (95% CI 0.98-1.56, P=0.09)]. No statistically significant interactions between any of the potentially effect-modifying factors (age, sex, smoking, body
mass index, alcohol consumption, hypertension, cholesterol levels and education) and antibody status were noted in the prediction of all-cause mortality.

Coeliac autoantibody-positive individuals evinced an increased risk of lymphoma as cause of death, this albeit based on few cases (N=2, Table 3 in III, Figure 3). Furthermore, a statistically significantly increased risk of stroke and diseases of the respiratory system as well as dementia was detected in Celikey tTG-positive subjects. Except for dementia, the risk estimates in EMA-positive subjects were parallel with those in Celikey tTG-positive cases (Figure 3, Table 3 in III).
Figure 2. Age and sex adjusted risks (hazard ratios) of different cancers between persons with positive and negative endomysial (EMA, black circles) and tissue transglutaminase antibodies (Celikey tTG, open circles). Risks of certain malignancies are not reported due to the low number of diseased cases in antibody-positive individuals.
Figure 3. Age and sex adjusted risks (hazard ratios) of all-cause and cause-specific mortality between persons with positive and negative endomysial (EMA, black circles) and tissue transglutaminase antibodies (Celikey tTG, open circles). Certain risks are not reported due to the low number of specific deaths in antibody-positive individuals.

1 The analysis was repeated after exclusion of cases with the mentioned illness at the beginning of follow-up, and the results remained virtually the same.
2 Exclusion of cases with the defined illness at the beginning of follow-up was not possible.
6. DISCUSSION

6.1 Methodological considerations

The result of any study may be due to bias, confounding or chance or it may reflect a truth. This section addresses any potential for the current study to yield fallacious results.

Due to the similar sampling methods applied to the whole Finnish population aged 30 years and over, the Mini-Finland (1978-80) and Health 2000 (2000-01) surveys have produced extensive data for cross-sectional studies as well as for comparative analyses between the study cohorts (Aromaa et al. 1989, Aromaa and Koskinen 2004). Further, the similar serological methods and the uniform diagnostic criteria used to detect all coeliac disease cases in both cohorts confirmed the comparability of prevalence figures between the surveys in the present study (I).

6.1.1 Selection bias

The high participation rates of the Mini-Finland (90%) and Health 2000 (84%) population-based surveys are crucial for the validity of the results of the current study. Sociodemographic characteristics between participants and non-participants in the surveys were not markedly different (Aromaa et al. 1989, Aromaa and Koskinen 2004, Heistaro 2005). The dropout rate varied slightly from group to group in both surveys, i.e. participation decreased somewhat with age and a slightly smaller proportion of the oldest women took part than men (Aromaa et al. 1989, Aromaa and Koskinen 2004, Heistaro 2005). Since the non-participants in both surveys resembled each other, the comparison of the prevalence figures is not likely to have been distorted, and in fact drop-out rates were so small as to be unlikely to affect the validity of findings. Due to the unavailability of some sera for analyses in the present study the participation rates were slightly decreased, being still nonetheless 87% in the Mini-Finland and 80% in the Health 2000 surveys. The
missingness of sera was apparently independent of coeliac disease, thus not inductive of selection bias. There is therefore no reason to believe that non-participants differed from participants by indicators connected to coeliac disease.

Undetected coeliac disease was defined by two-stage serological screening applied to all participants, i.e. all sera were tested for Eu-tTG in the first stage of screening and only sera positive for Eu-tTG were further analysed for Celikey tTG and EMA. The screening method enabled the finding of undetected cases in the present study. When assessing the prognosis of unrecognized coeliac disease, it was also possible to avoid selection bias due to ascertainment. However, survival bias could not be excluded, as it was possible to study only prevalent cases of unrecognized coeliac disease. In theory, the bias might lead to selection of cases with a milder clinical picture and thus spurious reduction of the risk. Potential changes in survival of coeliac disease patients over time may also have affected the populations of unrecognized coeliac disease two decades apart. If the overall risk of mortality in unrecognized coeliac disease had decreased over time, patients with a long course of disease would have been under-represented in the Mini-Finland survey (1978-80) compared to the survey in 2000-01. This might have falsely emphasized the difference in the total prevalence of coeliac disease two decades apart (I). However, the cohorts included partially the same generations and thus scarcely differed enough to explain the doubling of the total prevalence of coeliac disease. Furthermore, as the outcome data were extracted from the nationwide registers, the risk of selection bias as a consequence of loss to follow-up would appear low.

All in all, mainly due to excellent participation rates, thorough screening of all participants and low risk for loss to follow-up, the risk of selection bias seems to have remained low in the current study.

### 6.1.2 Information bias

In addition to enquiry whether participants in both surveys had any chronic disease, the participants in the Health 2000 survey were also asked separately whether they had coeliac disease. In theory, some diagnosed coeliac disease cases in the Mini-Finland survey may have remained unnoticed due to the rather unspecific question
posed, this possibly increasing the difference in prevalence figures between the cohorts. In practice, as coeliac disease affects the every-day life of patients, it is unlikely that individuals with the condition would not report it when any chronic diseases are asked after. Further, the validity of the diagnoses was improved, as reported coeliac disease or dermatitis herpetiformis was scrutinized by case report data. To be able to compare the prevalence of diagnosed disease between the cohorts the same diagnostic criteria were used in both surveys.

The definition of unrecognized coeliac disease was based on positive test results for both Eu-tTG and either EMA or Celikey tTG. Despite good but not perfect specificity of the test for EMA (Table 3, page 23), there is still a theoretical possibility of false-positive cases of unrecognized coeliac disease. However, the finding of a real false-positive case is most probably a rarity attributable to the patchiness of mucosal pathology (Rostom et al. 2005) and further, EMA-positive cases without manifest mucosal lesion have been shown to be gluten-sensitive and to evince villous atrophy later in life (Egan-Mitchell et al. 1981, Mäki et al. 1990, Collin et al. 1993, Kaukinen et al. 2005, Salmi et al. 2006, Kurppa et al. 2009). High validity of the test for tTG-ab has also been reported and its sensitivity seems even to outweigh that of the test for EMA (Tables 3 and 4, pages 23 and 24). In the current study the two-stage screening algorithm was used to optimize sensitivity in the first step and specificity in the second, thus enabling identification of the majority of patients with unrecognized coeliac disease with a minimum of falsely seropositive individuals. This was especially important in the sera of the Mini-Finland survey, as Eu-tTG yielded an 8% prevalence of positive cases.

To assess the prognosis of unrecognized coeliac disease a retrospective study design with stored sera seemed to be the only practical mode of approach. A prospective study design with untreated diagnosed coeliac disease patients would have involved ethical problems and further, would not have yielded results in reasonable time. Estimation of the total prevalence of coeliac disease in the past was likewise not possible without stored sera. Despite the widespread use of banked serum specimens, there is a paucity of data available regarding the effect of storage on the measured antibody result. It is conceivable that the storage and freeze-thaw cycles may damage protein structures, possibly leading to decreased levels of measured protein (Petrakis 1985, Evans et al. 1996). On the other hand, dessication of a specimen might lead to a concentration of samples (Petrakis 1985) and
increased levels of proteins with extended storage time (Hostmark et al. 2001, Männistö et al. 2007). However, several constituents of the blood have been shown to remain stable at subfreezing temperatures during long-term storage (Petrakis 1985, Pagani et al. 1998, Linneberg et al. 2000) and after repeated freeze-thaw cycles (Pinsky et al. 2003). As to the stability of tTG-ab and EMA in the current study, IgA has been reported to be stable at -20°C for indefinite periods (Petrakis 1985, Rubio-Tapia et al. 2009). Thus, the high Eu-tTG positivity rate is most probably due to concentration of the samples from the Mini-Finland cohort (1978-80), resulting in an increased optical density in the ELISA and hence many low positive cases. Firstly, such a conception is supported by the low positivity in Eu-tTG-positive cases in the Mini-Finland cohort (median value 8.4 AU/ml, lower quartile 7.5, upper quartile 10.0, range 7.1-25.0) when compared with the more recent Health 2000 cohort (median value 16.2 AU/ml, lower quartile 9.9, upper quartile 21.0, range 7.1-26.0). Secondly, the influence of storing seemed also not to be random, as none of the Eu-tTG negative sera were Celikey tTG- or EMA-positive (Figure 1 in II). Thirdly, the high yield of Eu-tTG was taken into account in the design of the study, as only cases with positive test result for both Eu-tTG and either EMA or Celikey tTG were defined as having unrecognized coeliac disease.

Since the tests for EMA and tTG-ab measure the same antibody in sera, it would be logical to think that the possible influence of storing on both antibodies would be parallel, i.e. reducing specificity and increasing sensitivity. At least in theory, the decreased specificity of EMA might have increased the estimated prevalence of coeliac disease in 1978-80 and thus, erroneously reduced the difference in the total prevalence of coeliac disease between the cohorts two decades apart. However, this was hardly the case, as the yield of the EMA test was much lower (1.0%) compared to Eu-tTG (8.3%) and Celikey tTG (2.9%) in the old sera gathered in 1978-80. In the prognostic studies, decreased specificity might have led to dilution of a real effect especially in tTG-ab-positive cases.

Illogical as it might seem, one may still wonder whether the influence of storing on EMA could be the opposite compared to tTG-ab, i.e. reducing sensitivity and increasing specificity. Basically the high titres of EMA in the Mini-Finland cohort (only five EMA-positive cases with titres 1:5) do not bespeak the degradation of the antibodies over time. Further, all separate EMA-positive sera remained positive after years of storage.
Validity of the conclusions reached in the present study also depends on the quality of the registers used, the Finnish cancer registry and the causes of death register. The Finnish cancer registry is recognized for its completeness and accuracy, as it includes >99% of incident cases of any malignancy diagnosed in Finland since 1953 and has low false-positive and false-negative discrepancy rates (Teppo et al. 1994, Korhonen et al. 2002). As to the cause of death register, it has likewise good coverage, as all deaths in Finland since 1936 are included (Statistics Finland) and good validity of its data has also been reported (Mähönen et al. 1999).

6.1.3 Confounding factors

Confounding factors are present when the relationship between the exposure and outcome of interest is mixed with the effect of another exposure on the same outcome and, further the two exposures are correlated. In addition, confounding factors should not be present in the causal pathway between the studied exposure and outcome. Comprehensive data collection in the Mini-Finland and Health 2000 surveys in the past enabled control of different confounding variables such as education, body mass index and smoking. The methods used to control confounding elements in analysis in the present study comprised stratification as well as adjustment by multivariate techniques (dos Santos Silva 1999).

6.1.4 Random error

The result of the present series is only an estimate of the phenomenon studied in the source population, as random error leads to lack of precision in occurrence or effect measures. Both hypothesis testing and estimation of confidence intervals (CI) were used to evaluate the effects of random error on the results (dos Santos Silva 1999).

Although the sample of EMA- and Celikey tTG-positive cases was large enough to assess the prevalence of coeliac disease over time as well as the overall risks of mortality and malignancies, the numbers were potentially too small for some of subgroup analyses, this leading to rather wide 95% CIs.
6.1.5 Generalizability and causality

The two-stage large samples of the Mini-Finland and Health 2000 surveys were representative of the whole Finnish population aged 30 and over, allowing for good generalizability of the results at least as regards Finnish adults. With certain reserves, the increase in the total prevalence of coeliac disease as well as the results of the prognostic studies may also be generalized to other Western countries.

Even though the influence of bias, confounding factors and chance could theoretically be minimized, the observed association would still not necessarily be causal. In the current study it is reasonable to evaluate the associations found between unrecognized coeliac disease and specific malignancies as well as causes of death under Hill’s criteria for causality (Hill 1965). The nine criteria in question are temporal relationship, strength of association, consistency, specificity, exposure-response relationship, biological plausibility, coherence, reversibility and analogy. The only criterion regarded as essential for causality is that an exposure variable precede the outcome variable, which is the case in the present follow-up studies. The criterion of specificity was not fulfilled, as coeliac disease, like the majority of other chronic diseases, is not associated with one specific condition but many diseases. Due to the relatively low number of autoantibody-positive unrecognized coeliac disease cases with defined outcome, the evaluation of an exposure-response relationship was not possible in the evaluation of causality in the reported associations. Other areas of causality are discussed later within the scope of available evidence.

In summary, the limitations discussed above need to be considered when interpreting the results of the present study.

6.2 Increasing prevalence of coeliac disease over time

The prevalence of clinically diagnosed coeliac disease increased substantially during the period covered by the present study. The prevalences in 1978-80 (0.03%) and in 2000-01 (0.52%) are well in line with those in other reports from Finland and Europe (Greco et al. 1992, Collin et al. 1997a, Collin et al. 2007, Vilppula et al. 2008). In the late 1970s coeliac disease was considered a rare disorder affecting
approximately 1 per 1,000 (0.1%) individuals, mostly of European origin (Greco et al. 1992). Subsequently the clinical prevalence was reported in Finnish adults to be 0.27% in 1994, 0.45% in 2003 and 0.55% in 2006 (Collin et al. 1997a, Virta et al. 2009). When elderly Finns were considered separately, the clinical prevalence was as high as 0.89% in 2002 (Vilppula et al. 2008). The increasing figures of clinically diagnosed cases were most probably due to a better awareness of the disease and improved diagnostic facilities such as serological screening tests, as well as the availability of endoscopy with routine small-bowel biopsy.

Regardless of better diagnostics and thus the increased numbers of coeliac disease patients over time seen in the present study, the number of EMA-positive unrecognized cases also increased in a statistically significant manner, being 1.03% in 1978-80 and 1.47% in 2000-01. The results are well in line with those of the first screening studies in Europe, whereby the seroprevalence of EMA has varied between 0.2% and 1.2% (Table 5, page 32). The present study further revealed that up to 74% of all coeliac disease cases still remained undiagnosed in 2000-01. Only recently the highest prevalence (3%) ever reported in any population in Europe or North America, was confirmed in Swedish children and further, two thirds of the cases in question had also remained undiagnosed before the screening programme (Myleus et al. 2009). Further, other screening studies in Finland have also uncovered a number of undetected cases, as none of the diseased children and only roughly 40% of the elderly had received a proper diagnosis before the implementation of the studies (Mäki et al. 2003, Vilppula et al. 2008, Vilppula et al. 2009). Challenges in diagnostics such as the ability of the conventional serological tests to find IgA-deficient cases as well as that of small-bowel biopsy to find patchy lesions still prevail. However, the main reasons for underdiagnosis are most probably the diverse clinical picture and the presence of virtually asymptomatic cases.

The main finding was an increase in the total prevalence of coeliac disease in Finnish adults over time, as it nearly doubled from 1.05% (1978-80) to 1.99% (2000-01) during two decades. Comparable prevalence figures including both diagnosed and previously unrecognized antibody-positive cases have been reported in Finnish children (1.53%) as well as in the elderly (2.70%) (Mäki et al. 2003, Vilppula et al. 2008, Vilppula et al. 2009). Although the increase in the total prevalence over time was first reported in the present study, a similar increase was
later confirmed in the population of the USA. There the prevalence of unrecognized coeliac disease was defined by two-stage serological screening comparable to that used in the present study and it was 0.2% in 1948-54 compared to 0.9% in 2006-08 in a mainly male American population.

The increased coeliac disease prevalence in type 1 diabetic children in the course of time may further support the reported increase in the total prevalence of coeliac disease (Mäki et al. 1984, Hansen et al. 2006, Salardi et al. 2008). According to an early report only 2% of children with type 1 diabetes had coeliac disease (Mäki et al. 1984) compared to the roughly 10% established recently (Hansen et al. 2006, Salardi et al. 2008). Furthermore, the findings that the highest EMA prevalences so far have been detected in children (Tables 5 and 6, pages 32-35) and that substantial seroconversion may take place in adulthood (Vilppula et al. 2009) are in accord with the conception of an increasing prevalence of coeliac disease over time.

While the present study could show for the first time that the total prevalence of coeliac disease had increased in the course of time, the same phenomenon has been shown in other autoimmune diseases and allergy. Type 1 diabetes has become more common in Finland since the 1950s (Figure 2 in I) and its incidence continues to increase widely in Europe (Harjutsalo et al. 2008, Patterson et al. 2009). In addition, the incidence of multiple sclerosis and Crohn’s disease as well as allergic diseases has also increased (Woolcock and Peat 1997, Bach 2002, Warren et al. 2008, Grieci and Butter 2009).

Given the relatively short time interval in question, environmental factors rather than genetic changes seem the more likely explanation for the increase. So far, research in the field of environmental factors affecting coeliac disease has focused on infant feeding practices such as consumption of cereals and breastfeeding. As early as in the 1950s the beneficial effect of breastfeeding on the onset of coeliac disease was first suggested (Jones et al. 1964) and according to a meta-analysis based on several case-control studies, the risk of coeliac disease was significantly reduced in infants breastfed at the time of gluten introduction (Akobeng et al. 2006). However, the protective effect of breastfeeding could not subsequently be reported in a prospective cohort study (Norris et al. 2005). In addition to breastfeeding, gluten introduction may have an effect on the onset of coeliac disease. Low intake of gluten-containing cereals (Ascher et al. 1993, Ivarsson et al. 2000, Ivarsson et al. 2002) as well as age at introduction of gluten-containing cereals at four to six
months (Ivarsson et al. 2000, Norris et al. 2005) could possibly prevent the development of the disease. However, further larger studies are needed to confirm the findings and it is not known whether infant feeding patterns merely delay the clinical expression of coeliac disease and do not affect the underlying process which results in the small-intestinal coeliac lesion. Proportions of women still breastfeeding their babies at the age of six months (Verronen 1988) as well as the amount of wheat consumed (Salovaara 1979, Leppälä 1992) have changed in Finland over time and might partly explain the increased prevalence figures. It has also been suggested that other dietary factors such as the amount of gluten-containing cereals in later life, different quality and varieties of wheat and type of cow’s milk formula could make a contribution to the risk of coeliac disease (Cronin and Shanahan 2001, Fasano and Catassi 2001).

Other possible environmental factor responsible for increasing prevalence figures might be the lack of an adequate number of infections in early childhood. This so-called hygiene hypothesis has been postulated to explain the increased prevalence of both allergic and autoimmune disorders in industrialized countries and could therefore in due course account for increase in the total prevalence of coeliac disease, too (Bach 2002, Rautava et al. 2004). The hypothesis suggests that environmental changes in the industrialized world have led to reduced microbial contact at an early age, thus up-regulating immunity and resulting in a growing epidemic of allergic and autoimmune disorders. The possible environmental factors affecting the number of infections might be socioeconomic circumstances such as income, day-care, number of siblings, nutrition, climate, level of medical care and use of antibiotics (Bach 2002). The hypothesis is strongly supported by numerous studies on both humans and animals; e.g. probiotics have been shown to be beneficial in atopic dermatitis (Kirjavainen et al. 2002) and further, by infecting nonobese diabetic mice with different microbes type 1 diabetes has repeatedly been prevented (Takei et al. 1992, Cooke et al. 1999). As to coeliac disease, a five-fold prevalence of biopsy-proven disease has been shown in Finland compared to the adjacent population of Russian Karelia (Kondrashova et al. 2008). This is line with the previous observation that Finland also had a six-fold incidence of type 1 diabetes compared to Karelia (Kondrashova et al. 2005). Environmental factors such as inferior prosperity and standard of hygiene in Russian Karelia compared to Finland
were suggested to be in the background (Kondrashova et al. 2005, Kondrashova et al. 2008).

Finally, it may be hypothesized that possible changes in other environmental factors and living habits over time might account for the change in the total prevalence of coeliac disease. However, studies concerning the issue are sparse and the results are not convincing. For example, the effect of tobacco use on the risk of coeliac disease seems to be a matter of controversy (Prasad et al. 2001, Veldhuyzen van Zanten 2001, Austin et al. 2002, Suman et al. 2003, West et al. 2003) and adjustment for smoking had no substantial effect on the risk of coeliac disease in the present study. Further, the suggestion that heavy drinking could lead to the initiation of an autoimmune response against tissue transglutaminase should still be confirmed in future studies (Koivisto et al. 2008).

6.3 Prognosis of undetected coeliac disease as regards malignancies

According to the present findings no increased risk of overall malignancy was detected in previously unrecognized coeliac autoantibody-positive cases. As the current study was the first to address the question and corresponding studies have not hitherto been published, it is necessary to compare the present results to previous reports on the risk of malignancies in diagnosed coeliac disease and dermatitis herpetiformis patients (Table 7, page 40). In conflict with the oldest studies showing an increased risk of overall malignancy in coeliac disease, the majority of recent papers do not report increased cancer risk. As to the risk in dermatitis herpetiformis, the highest risks can also be seen in the oldest studies and at maximum of 1.2-fold risk in recent reports. In addition to any bias such as ascertainment and positive result bias, an explanation for the highest cancer risk in the oldest studies might also be due to dissimilar study populations, followed by changing diagnostic activity over time. The suspicion of coeliac disease at the time of the earliest studies was based mainly on clinical symptoms such as diarrhoea, malabsorption and weight loss, while in recent years, following improvements in diagnostics, more and more coeliac disease patients with mild or absent symptoms have most probably been included in the study settings. The hypothesis here is that
when only the most serious cases are included, the risk of overall malignancy is increased, but when study cohorts comprise cases with both serious and mild clinical picture, the risk no longer exists. The hypothesis can also be extended to the present study, where the study population of unrecognized coeliac disease cases with positive autoantibody status was not dependent on severity of symptoms. Thus, the majority of these cases may have suffered from a mild clinical state or they may also have been apparently asymptomatic, this explaining the good prognosis as regards the overall risk of malignancies. Early diagnosis of coeliac disease and thus early commitment to a gluten-free diet might also prevent complications such as malignancies (Holmes et al. 1989, Silano et al. 2008, Gao et al. 2009). However, only few coeliac disease cases in the Mini-Finland cohort had presumably received a proper diagnosis in surveillance and thus, a gluten-free diet hardly had a major role as regards good prognosis of unrecognized coeliac disease cases in the current study. Nonetheless, in contrast to the findings in this series, tTG-positive cases have been shown to have a 3.6-fold excess of cancer as the cause of death during eight years of follow-up (Metzger et al. 2006). The study in question had a male predominance, which raises the question whether antibody-positive individuals actually represented coeliac disease cases.

Despite good prognosis as regards the overall risk of malignancies, the present results suggest that the risk of specific malignancies, i.e. NHL and carcinoma of the oesophagus, might be increased in unrecognized coeliac disease. As the risk estimates are based on relatively few cases, the effect of chance cannot be ruled out. However, an increased risk of lymphoma in diagnosed coeliac disease populations has repeatedly been shown over the last few decades, although the risk seems to be highest in the oldest and studies comparable to a decreasing trend over time when estimating the overall risk of malignancies (Table 8, page 44). Three-to six-fold risks of NHL in EMA-and Celikey tTG–positive cases in the present study are fully comparable to recent results in diagnosed coeliac disease and dermatitis herpetiformis (Table 8, page 44). Thus, previous reports support the finding in the current study, even though the number of unrecognized coeliac disease cases with NHL in a follow-up of nearly 20 years remained low. The association between NHL and unrecognized coeliac disease has also been clarified in a case-control study, where the occurrence of both previously diagnosed and screen-detected coeliac disease was evaluated in consecutive patients with newly diagnosed NHL and
control individuals (Mearin et al. 2006). Interestingly, an increased risk could only be found in previously clinically diagnosed patients and not in the subgroup of previously unrecognized cases. However, due to the relatively low number of screen-detected individuals in the study, the 95% CI remained wide (Mearin et al. 2006). In other corresponding case-control studies either unrecognized cases were not searched for at all or the number of such cases was far too low to draw conclusions (Catassi et al. 2002, Farre et al. 2004, Smedby et al. 2006, Gao et al. 2009). The mechanisms explaining the increased risk of NHL in coeliac disease remain unknown. Nor is it known whether there is a causal relationship between the diseases. However, an increased risk of NHL has been shown in persons who have a sibling affected by coeliac disease (Gao et al. 2009). It has thus been suggested that coeliac disease and NHL could share the same genetic risk factors (Gao et al. 2009). Nor can environmental factors inducing both illnesses be ruled out.

Additionally, the risk of carcinoma of the oesophagus was also increased in Celikey tTG but not in EMA–positive individuals in the current study. While the result remains to be confirmed, diagnosed coeliac disease cases have been shown to carry an increased risk of gastrointestinal cancers and especially malignancies of oropharynx, oesophagus and small intestine in some study settings (Holmes et al. 1976, Selby and Gallagher 1979, Holmes et al. 1989, Askling et al. 2002, Green et al. 2003, Silano et al. 2007). However, the association with gastrointestinal cancers has not been shown in all studies concerning the issue (Card et al. 2004, West et al. 2004a, Viljamaa et al. 2006, Goldacre et al. 2008). While inflammation of the small-bowel mucosa in coeliac disease is well recognized (Marsh 1992) the mucosa of the whole upper gastrointestinal tract might be damaged (Oderda et al. 1993) and could, at least in theory, induce carcinogenesis. However, it has to be borne in mind that the most prominent risk factors for squamous cell carcinoma of the oesophagus in the Western world are alcohol and tobacco use (Ribeiro et al. 1996) and the main established risk factors for adenocarcinoma of oesophagus are Barrett’s oesophagus, gastro-oesophageal reflux, and obesity (Lagergren 2005). Regardless of the results of the present study, an increase in the risk of certain uncommon specific malignant conditions such as enteropathy-associated T-cell lymphoma in unrecognized coeliac disease cannot be ruled out.
6.4 Prognosis of undetected coeliac disease as regards mortality

According to the current findings, no statistically increased risk of all-cause mortality was established among coeliac antibody-positive undetected coeliac disease cases. This is in contrast to the increased risk of overall mortality previously reported in clinically diagnosed coeliac disease (Table 9, page 48). In addition to the difference in target group, the participants in the current study have been sampled from the general population, in contrast to a majority of previous studies. Further, the risk of mortality in dermatitis herpetiformis has been comparable to that in the general population and is thus well in line with the risk carried by unrecognized cases detected in the present study. As already noted in the previous section on malignancies, the undetected coeliac disease population in the present study was found by serological screening independent of symptoms and many coeliac disease cases included were thus most probably apparently asymptomatic or evinced a mild clinical picture, this potentially explaining the different risk levels between diagnosed and unrecognized coeliac disease cases. The unrecognized coeliac disease population in the present study might mimic dermatitis herpetiformis patients as regards severity of symptoms and histology and could therefore explain the similar risk of overall mortality in both groups (Reunala et al. 1984, Fry 2002).

As to the borderline significant modestly elevated risk (19%) of overall mortality in Celikey tTG–positive cases, it is not possible to conclude whether this might reach statistical significance in still larger settings. However, the risk estimate seems to be relatively low.

Only few studies have concentrated on the association between coeliac autoantibody positivity, most probably representing unrecognized coeliac disease, and mortality (Johnston et al. 1998, Metzger et al. 2006, Rubio-Tapia et al. 2009). Metzger and associates (2006) compared screen-detected tTG-ab-positive individuals from the general population (n=63) to negative cases as regards mortality over a period of eight years and found a 2.5–fold increased risk (95% CI 1.5-4.3). As males predominated in the study, the question arises whether antibody-positive cases in the study represented coeliac disease. Further, a recent cohort study with roughly 9,000 participants from the American Air Force revealed a 3.9-fold risk of all cause mortality in undetected coeliac disease defined by serology (Rubio-
Tapia et al. 2009). The study population in question consisted mainly of males and, only 14 of the participants proved to have undetected disease and nine of them died during the surveillance. In contrast, Johnston and associates (1998) found no an increased risk of mortality in coeliac antibody-positive individuals compared to negative subjects (Johnston et al. 1998). However, the weakness of the study was that besides their use of rather unspecific IgA antigliadin antibodies they also grouped the participants as antibody-positive if any IgA-class antigliadin, antireticulin or antiendomysium autoantibodies were positive. This result is in line with the follow-up of cases found by risk-group screening (Corrao et al. 2001) and mass-screening (West et al. 2003), but unfortunately the analyses had only limited power, as one death in the former and five deaths in the latter study cohort were observed. As to the recent large study by Ludvigsson and colleagues (2009), 1.3-fold risk in mortality in latent coeliac disease (positive coeliac disease serology in individuals with normal mucosa) was reported. When only latent cases with either EMA or tTG-ab were included in the analysis, an increased risk was no longer reported. However, the cases with latent coeliac disease were not screened from the general population and thus differ substantially from the participants in the present study, where a population-based sample was screened independent of symptoms.

Overall risk of malignancies was not increased as cause of death, which is in line with the findings of the present study that no increased risk of cancer morbidity was detected. However, unrecognized cases in the current study carried an increased risk of NHL. The debate on these issues has already been addressed in the previous section.

Undetected coeliac disease in the present study also carried increased risk of stroke and diseases of the respiratory system as cause of death. As to diseases of the circulatory system, the association with coeliac disease is unclear. One might assume that the risk of cardiovascular diseases might be reduced in coeliac disease, as a favourable cardiovascular risk profile has been connected with the disease (West et al. 2003, West et al. 2004b). Earlier unrecognized EMA-positive cases were slightly lighter, they smoked less and their mean serum cholesterol was lower compared to non-coeliac individuals. Diagnosis of hypertension and hypercholesterolaemia has also been reported to be less likely in diagnosed cases (West et al. 2004b). On the other hand, malabsorption of folic acid followed by hyperhomocysteinaemia might lead to an excess risk of diseases of the circulatory
system (Homocysteine Studies Collaboration 2002, Dickey et al. 2008, Milani and Lavie 2008) and explain the increased risk of stroke as cause of death in undetected cases in the current study. As to the diagnosed cases, increased risks of circulatory system diseases have been reported (Ludvigsson et al. 2007a, Wei et al. 2008). In the largest study so far (Ludvigsson et al. 2009) the increase seems also to reflect causes of death, in contrast with smaller studies (Logan et al. 1989, Corrao et al. 2001, Viljamaa et al. 2006, Anderson et al. 2007). Only two studies have reported an association between diagnosed coeliac disease and cerebrovascular diseases as cause of death, with contrasting results (Viljamaa et al. 2006, Solaymani-Dodaran et al. 2007).

The observation that respiratory system diseases as cause of death were also overpresented in coeliac autoantibody-positive unrecognized cases is in line with findings in previous studies (Corrao et al. 2001, Peters et al. 2003, Viljamaa et al. 2006, Ludvigsson et al. 2009). Although there are only few studies addressing the association between coeliac disease and respiratory system diseases, some specific illnesses such as tuberculosis (Ludvigsson et al. 2007d), sarcoidosis and lung cavities independent of aetiology (Douglas et al. 1984, Stevens et al. 1990) have been held to be associated with coeliac disease. Any disturbances in immune function in coeliac disease might also predispose individuals to diseases of the respiratory system.
7. CONCLUSIONS AND FUTURE ASPECTS

In conclusion, the current study emphasized the importance of coeliac disease as an emerging health problem, as the total prevalence was shown to have increased and nearly doubled over two decades in the recent past. Further, regardless of improved diagnostics, the proportion of undetected condition proved to be high, embracing even three quarters of the diseased population. The overall risks of malignancies and mortality in the undetected condition compared to the general population was not increased in the long-term follow-up studies. Nonetheless, specific malignancies, i.e. NHL and carcinoma of the oesophagus, seemed to be associated with the condition. Although the observation is in line with the risk profile in diagnosed cases, the finding, as well as increased risks of stroke and diseases of the respiratory tract as cause of death, must be confirmed in still larger forthcoming studies.

Substantial challenges for the future still remain in the field of coeliac disease research. It is clear that coeliac disease extends over all continents with surprisingly high, roughly 0.5-2%, prevalence figures in recent years. The present study further underlined the significant increase in the total prevalence of the disease over time. It remains to be seen, whether the same increasing trend will continue and whether environmental factors responsible for the increasing prevalence figures will be indentified. By means of forthcoming studies concentrating on these issues, even prevention of coeliac disease might become possible.

The present study also revealed that up to three-quarters of the coeliac disease population still remained unrecognized at the beginning of the 21st century. This raises the question whether all coeliac disease cases should be diagnosed and treated at an early stage this implying implementation of population screening programmes. The criteria for screening programmes set out by the World Health Organization (Wilson 1968), for example recognizable pre-clinical state, available screening test and diagnostic facilities (Walker-Smith et al. 1990, Rostom et al. 2005, Lewis and Scott 2006) as well as acceptable treatment, are fulfilled in coeliac disease at least in
industrialized countries. On the other hand, the natural history of undetected coeliac disease is not fully known. The present series substantially clarified the issue, as no increased risk of overall mortality or malignancies was found in coeliac autoantibody-positive previously undetected individuals. However, three- to six-fold risks of NHL were detected in the unrecognized condition, the same risk level as previously reported in diagnosed cases. Presuming causality between coeliac disease and NHL, the population-attributable fraction can be calculated. In subjects with diagnosed coeliac disease the fraction has been estimated to be 0.1% (Gao et al. 2009, West 2009). As roughly 1,000 patients with NHL are diagnosed yearly in Finland (Finnish cancer registry), only one of them could be prevented per year if the onset of coeliac disease had never occurred. If, further, the undetected condition had been taken into account, the number of potentially preventable NHL would most probably have increased, remaining nonetheless at a low level. In summary, the findings of the present study do not support implementation of population screening programmes. It is tempting to cite the editorial of Gut (Logan 2009) and say that good prognosis of undetected coeliac disease as regards mortality and malignancies might be a nail in the coffin for mass screening.

Apart from a need for confirmatory studies regarding mortality and malignancies in different settings, forthcoming work should still focus on the natural history of undetected disease concerning other important outcomes such as fractures, autoimmune diseases and quality of life. If any health risk in the undetected condition is to be found, the efficacy of population screening to improve the prognosis of diseased individuals as well as the costs should still be clarified.
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Tampere, December 2009

Sini Lohi
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ORIGINAL PUBLICATIONS
Increasing prevalence of coeliac disease over time

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SUMMARY

Background
The number of coeliac disease diagnoses has increased in the recent past and according to screening studies, the total prevalence of the disorder is around 1%.

Aim
To establish whether the increased number of coeliac disease cases reflects a true rise in disease frequency.

Methods
The total prevalence of coeliac disease was determined in two population-based samples representing the Finnish adult population in 1978–80 and 2000–01 and comprising 8000 and 8028 individuals, respectively. Both clinically–diagnosed coeliac disease patients and previously unrecognized cases identified by serum endomysial antibodies were taken into account.

Results
Only two (clinical prevalence of 0.03%) patients had been diagnosed on clinical grounds in 1978–80, in contrast to 32 (0.52%) in 2000–01. The prevalence of earlier unrecognized cases increased statistically significantly from 1.03% to 1.47% during the same period. This yields a total prevalence of coeliac disease of 1.05% in 1978–80 and 1.99% in 2000–01.

Conclusions
The total prevalence of coeliac disease seems to have doubled in Finland during the last two decades, and the increase cannot be attributed to the better detection rate. The environmental factors responsible for the increasing prevalence of the disorder are issues for further studies.

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INTRODUCTION

Coeliac disease, which is induced by ingestion of cereal gluten, is a chronic autoimmune-mediated disease with both intestinal and extraintestinal manifestations. Until the late 1970s, the suspicion of coeliac disease was based mainly on clinical symptoms such as diarrhoea, malabsorption and weight loss. The disease was considered to be rare; the prevalence was estimated to be as low as 0.03% worldwide. Subsequently, the disease has been found more frequently in adults suffering from a variety of atypical symptoms and even in asymptomatic subjects. With realization of the diversity of its manifestations and the advent of highly sensitive and specific serological tests, endomysial and tissue transglutaminase antibody assays, the increasing trend in incidence figures could be verified. Furthermore, the tests enabled mass-screening of populations, and the prevalence of the disease was soon found to be around 1% in both Europe and the United States.

The changed prevalence figures have sparked off debate as to whether the increasing prevalence of the condition reflects a true rise in prevalence in the course of time or whether it is due simply to the better detection rate. It is intriguing to speculate that such an increase could be a phenomenon parallel to that observed in type 1 diabetes, other autoimmune disorders and allergic diseases. To assess the prevalence of the disease over time, we defined it in two representative national population-based cohorts collected in 1978–80 and in 2000–01. Firstly, we determined the clinical prevalence of the disease in both cohorts and secondly, we screened the rest of the participants using highly sensitive and specific screening tools to identify unrecognized cases. By adding together the numbers of clinically diagnosed coeliac disease patients and the screen-detected previously unrecognized cases we arrived at the total prevalence of the disorder in the two cohorts collected two decades apart. Our hypothesis was that a true rise in disease prevalence is in fact under way.

MATERIALS AND METHODS

Study populations

The prevalence of coeliac disease was determined in two cross-sectional population cohorts representing the adult populations in Finland at two different time-points. The first sampling, the Mini-Finland Health Survey, was carried out in 1978–1980. Details of the study design and the baseline results are extensively reported elsewhere. In brief, a nationally representative sample of 8000 persons has been drawn from the population aged 30 and over by a stratified two-stage cluster sampling design planned by Statistics Finland. The study population was drawn from 40 areas in different parts of the country. The participants attended a health examination, which included interviews, questionnaires, drawing of blood samples and a clinical examination by a physician. The participation rate was 90% (n = 7217).

The second nationally representative population sampling designed by professional epidemiologists was carried out in 2000–2001. The basic data from this Health 2000 Survey have recently been published by The National Public Health Institute, and one of the goals of the survey was to determine changes in population health since 1978–80 by comparing health issues with the Mini-Finland Health Survey. In summary, the two-stage cluster sample of 8028 persons aged 30 or more was drawn from 80 health service districts throughout the country. The survey comprised interviews, questionnaires, measurements and clinical examinations principally similar to those in the Mini-Finland Survey of 1978–80. The participation rate was 84% (n = 6770).

A flow-chart of the present study is presented in Figure 1 and a comparison of the cohorts by several variables in Table 1. The non-participants did not markedly differ from the participants in socio-demographic characteristics in both surveys. According to our follow-up data no differences were detected between the participants and non-participants as regards mortality and morbidity. There is no reason to believe that non-participants differed from participants by indicators connected to coeliac disease.

All participants gave informed consent in both health surveys. The Ethical Committee of Tampere University Hospital approved the study protocol.

Assessment of coeliac disease

Previously diagnosed coeliac disease patients

All participants in the Mini-Finland Survey in 1978–80 were interviewed and asked whether they had any chronic diseases. Chronic disorders were also recorded in the course of the clinical examinations.
In the Health 2000 Survey, participants were asked by structured questionnaire whether a physician had previously diagnosed coeliac disease or dermatitis herpetiformis. The physician responsible for the clinical examination recorded all chronic diseases in the participants.

In 2004, we further scrutinized the reported diagnoses of coeliac disease and dermatitis herpetiformis of both cohorts by case record data.

As dermatitis herpetiformis with skin manifestations is one form of coeliac disease, 114 cases fulfilling the diagnostic criteria for coeliac disease or dermatitis herpetiformis were included. The criteria for coeliac disease were villous atrophy with crypt hyperplasia in a small-bowel biopsy specimen and clinical or histological recovery on a gluten-free diet. From the 1970s the diagnosis of dermatitis herpetiformis has been based on the demonstration of pathognomic granular IgA deposits in the dermal papillae by direct immunofluorescence examination, and prior to the development of this method on a typical clinical picture. Only the scrutinized cases fulfilling the above-mentioned diagnostic criteria for coeliac disease or dermatitis herpetiformis were used in numerators in the calculations of clinical prevalences.

### Screening of unrecognized coeliac disease cases

The previously collected blood samples were stored at −20 °C for later analysis. In the Mini-Finland survey, a total of 6993 (3771 females) serum samples were available for determination of coeliac disease antibodies. This compares with 6402 (3527 females) samples in the Health 2000 survey. These figures were used as denominators in calculating the prevalence of coeliac disease. The availability of sera reduced the excellent participation rates by 3–4% in both cohorts; selection of these subjects did not depend on issues related to coeliac disease and is not likely to influence the results. The age and sex distributions of the participants with available serum samples are given in Table 2.

### Table 1. The age- and sex-adjusted characteristics of the study participants in the Mini-Finland- and Health 2000 surveys

<table>
<thead>
<tr>
<th></th>
<th>Mini-Finland survey</th>
<th>Health 2000 survey</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males, %*</td>
<td>45.8</td>
<td>47.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean age, years†</td>
<td>51.0</td>
<td>52.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Higher education, %</td>
<td>11.5</td>
<td>28.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean serum cholesterol, mmol/L</td>
<td>6.9</td>
<td>5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>23.5</td>
<td>25.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean BMI, kg/m²</td>
<td>25.8</td>
<td>26.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any chronic illness‡, %</td>
<td>45.9</td>
<td>51.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary disease‡, %</td>
<td>10.2</td>
<td>7.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes‡, %</td>
<td>4.7</td>
<td>5.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Cancer, any‡, %</td>
<td>2.4</td>
<td>4.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Adjusted for age; † Adjusted for sex; ‡ Self-reported.

In the Health 2000 Survey, participants were asked by structured questionnaire whether a physician had previously diagnosed coeliac disease or dermatitis herpetiformis. The physician responsible for the clinical examination recorded all chronic diseases in the participants.

In 2004, we further scrutinized the reported diagnoses of coeliac disease and dermatitis herpetiformis of both cohorts by case record data.

As dermatitis herpetiformis with skin manifestations is one form of coeliac disease, cases fulfilling the diagnostic criteria for coeliac disease or dermatitis herpetiformis were included. The criteria for coeliac disease were villous atrophy with crypt hyperplasia in a small-bowel biopsy specimen and clinical or histological recovery on a gluten-free diet. From the 1970s the diagnosis of dermatitis herpetiformis has been based on the demonstration of pathognomic granular IgA deposits in the dermal papillae by direct immunofluorescence examination, and prior to the development of this method on a typical clinical picture. Only the scrutinized cases fulfilling the above-mentioned diagnostic criteria for coeliac disease or dermatitis herpetiformis were used in numerators in the calculations of clinical prevalences.

### Screening of unrecognized coeliac disease cases

The previously collected blood samples were stored at −20 °C for later analysis. In the Mini-Finland survey, a total of 6993 (3771 females) serum samples were available for determination of coeliac disease antibodies. This compares with 6402 (3527 females) samples in the Health 2000 survey. These figures were used as denominators in calculating the prevalence of coeliac disease. The availability of sera reduced the excellent participation rates by 3–4% in both cohorts; selection of these subjects did not depend on issues related to coeliac disease and is not likely to influence the results. The age and sex distributions of the participants with available serum samples are given in Table 2.
Altogether, we analysed 13 395 serum samples for IgA-class tissue transglutaminase antibodies (Eu-tTG umana IgA, Eurospital S.p.A, Trieste, Italy) in 2001–02. The test used is based on an enzyme-linked immunosorbent assay technique (ELISA) with human recombinant tissue transglutaminase as antigen. Pooled estimates of the sensitivity and specificity of a human recombinant-based test have been reported to be 98% in adult populations. Results are given in arbitrary units (AU) and the cut-off point for the test was 7.0 AU/mL according to instructions of the manufacturer. We further analysed tissue transglutaminase positive sera for IgA class endomysial antibodies using an indirect immunofluorescence method and a characteristic staining pattern at a serum dilution 1:25 was considered positive. Endomysial antibody-positive cases were considered to have unrecognized coeliac disease unless there was an earlier diagnosis of coeliac disease or dermatitis herpetiformis.

Due to the unexpectedly high percentage of tissue transglutaminase antibody positivity in sera in the Mini-Finland survey collected 22 years earlier, we also randomly selected 128 (one in 50) tissue transglutaminase antibody-negative serum samples and tested them for endomysial antibodies. In addition, to evaluate the stability of endomysial antibodies after long storage at −20 °C, we reanalysed 12 separate sera previously positive for IgA endomysial antibodies and drawn from biopsy-proven untreated coeliac disease patients an average of 14 (11 to 18) years earlier. The laboratory performing the reanalyses was blinded as regards the results of the primary analyses.

### Statistical analysis

The analyses were performed using SAS 8.02 (SAS Institute, Cary, NC, USA) and SUDAAN 9.0.0 statistical software (Survey Data Analysis, Research Triangle Institute, Research Park Triangle, NC, USA), which takes into account sampling weights and design effects. A logistic regression model was applied to estimate adjusted prevalences with 95% confidence intervals (CI) and odds ratios between the two surveys. In calculating the odds ratios, age, sex, educational level and survey were included in the models. P-values were computed using Satterthwaite F-test and a value <0.05 was considered statistically significant.

### RESULTS

#### Prevalence of previously diagnosed coeliac disease

The prevalence of diagnosed coeliac disease has increased substantially during the last two decades in Finland: only two ascertained coeliac disease cases had been diagnosed in 1978–80 (clinical prevalence of 0.03%, 95% CI 0–0.07) compared to 32 (0.52%, 95% CI 0.35–0.68) in 2000–01.

#### Prevalence of unrecognized coeliac disease

In the Mini-Finland survey (1978–80), altogether 578 (8.27%) out of all the 6993 analysed serum samples were tissue transglutaminase antibody-positive (median value 8.4 AU/mL, lower quartile 7.5, upper quartile 10.0, range 7.1–25.0); 12.80% (74, 53 females) out of 578 tissue transglutaminase-positive samples were also endomysial antibody-positive (Figure 1). The prevalence of unrecognized coeliac disease was thus 1.03% (95% CI 0.79–1.27). None of the 128 randomly selected tissue transglutaminase-negative samples was endomysial antibody-positive.

In the more recent population cohort (2000–01), tissue transglutaminase antibody positivity was found in
129 (2.02%) of the 6402 serum samples analysed (median value 16.2 AU/mL, lower quartile 9.9, upper quartile 21.0, range 7.1–26.0). The number of unrecognized coeliac disease cases with positive endomysial antibodies was 92 (57 females), yielding a screen-detected prevalence of 1.47% (95% CI 1.17–1.77). The age- and sex-adjusted odds ratio for the prevalence of unrecognized coeliac disease between the two study cohorts was 1.45 (1.06–1.99).

In all 12 separate sera drawn from biopsy-proven untreated coeliac disease patients up to 18 years earlier, the endomysial antibody result remained positive.

**Total prevalence of coeliac disease**

The total prevalence of coeliac disease increased in a statistically significant manner from 1.05% (two previously diagnosed + 74 unrecognized coeliac disease cases out of 6993 subjects) in 1978–80 to 1.99% (32 + 92 out of 6402) in 2000–01 (Table 3). The age- and sex-adjusted odds ratio for prevalence between the two study cohorts was 1.94 (95% CI 1.44–2.60). After further adjustment for educational level, the odds ratio was 1.56 (95% CI 1.12–2.18). The age-adjusted total prevalence increased from 0.65% (95% CI 0.41–0.89) to 1.65% (95% CI 1.16–2.14) in men and from 1.40% (95% CI 1.05–1.75) to 2.29% (95% CI 1.78–2.80) in women. The total prevalence of coeliac disease increased in a statistically significant manner in the age-groups 30–44 and 45–54 and the increasing trend could also be seen in older age-groups (Table 3). In addition, screening revealed that as many as 97% (74 out of 76) of coeliac disease cases were unrecognized in 1978–80 and 74% (92 out of 124) still in 2000–01.

**DISCUSSION**

The findings here indicate for the first time that the total prevalence of coeliac disease has increased in the course of time. In Finland, it almost doubled during the time-span examined, being 1.05% in 1978–80 and 1.99% in 2000–01, and the increase could be seen in both sexes and different age-groups. We took advantage of two large adult-representative population-based cohorts. The outstanding participation rates, the similar sampling and serological testing methods and the uniform diagnostic criteria for both cohorts greatly strengthen the validity of our conclusions.

We based the definition of unrecognized coeliac disease on positivity for serum endomysial antibodies without small-bowel biopsy, as has previously been done in large screening studies in USA and Europe.\(^6,8,9\) The test used here has been standardized and validated in Europe,\(^17\) and its specificity has been repeatedly reported to approach 100%.\(^2\) Theoretically, there is a possibility of false-positive cases in both cohorts. In practice, the finding of a real false-positive case is most probably a rarity for the following reasons. The patchiness of mucosal pathology may wrongly lead to exclusion of coeliac disease and a so called false endomysial antibody-positive case in fact indicates false-negative histology.\(^2\) In addition, endomysial antibody positive cases with normal villous structure often evince villous atrophy and crypt hyperplasia later in life.\(^20,21\) These patients without manifest mucosal lesion may even be gluten-sensitive, with a favourable response to gluten-free diet.\(^22–28\) Furthermore, a high concordance between endomysial antibody positivity and the coeliac type HLA-genotype, i.e. DQ2 or DQ8, has been clearly shown regardless of small-intestinal mucosal histology.\(^7,20,29\) As pooled sensitivity of endomysial antibodies has been reported to be 90% in adults,\(^2\) we cannot exclude the possibility that there were some endomysial antibody-negative coeliac disease cases in both cohorts. In such a case, our prevalence figures may even slightly underestimate the true prevalence at the defined time-points.\(^30\)

---

### Table 3. Total prevalence of coeliac disease in 1978–80 and 2000–01 according to age

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Mini-Finland year 1978–80</th>
<th>Health year 2000–01</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–44</td>
<td>1.06 (0.69–1.43)</td>
<td>1.87 (1.28–2.46)†</td>
<td>0.01</td>
</tr>
<tr>
<td>45–54</td>
<td>1.27 (0.68–1.86)</td>
<td>2.41 (1.57–3.25)†</td>
<td>0.03</td>
</tr>
<tr>
<td>55–64</td>
<td>1.28 (0.71–1.85)</td>
<td>2.20 (1.30–3.10)</td>
<td>0.08</td>
</tr>
<tr>
<td>65–74</td>
<td>0.84 (0.31–1.37)</td>
<td>1.68 (0.86–2.50)</td>
<td>0.1</td>
</tr>
<tr>
<td>75–</td>
<td>0.28 (0–0.83)</td>
<td>1.21 (0.35–2.07)</td>
<td>0.18</td>
</tr>
<tr>
<td>All</td>
<td>1.05 (0.80–1.29)</td>
<td>1.99 (1.64–2.33)†</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Sex-adjusted prevalences with 95% confidence intervals were estimated by a logistic regression model. Both earlier diagnosed coeliac disease patients and screen-detected endomysial antibody-positive cases were included in the prevalence figures; † The difference between the surveys is statistically significant.
We detected a surprisingly high frequency of tissue transglutaminase antibody positivity in the old sera collected in 1978–80. Tissue transglutaminase antibodies were not used in calculating the prevalence of coeliac disease, as this hardly represents the true prevalence of unrecognized coeliac disease in this study. As to the fact that tissue transglutaminase antibody tests have earlier yielded positive results in chronic liver and heart diseases without concomitant coeliac disease, the most likely explanation is the concentration of old sera, resulting in an increased optical density in the ELISA method, many low positive cases and hence a high positivity rate. Besides, to ascertain that most if not all unrecognized coeliac disease cases were among the tissue transglutaminase antibody-positive subjects, we randomly tested one in 50 tissue transglutaminase-negative individuals and showed that none was endomysial antibody-positive. In addition, long-term storage at -20 °C does not seem to affect sensitivity of IgA endomysial antibodies, as all separate sera drawn from biopsy-proven untreated coeliac disease patients with no severe symptoms up to 18 years earlier remained positive. It is also unlikely that sensitivity had declined because of decreased endomysial antibody titre during the storage, as in contrast, the proportion of tissue transglutaminase antibody positive cases was high defined from the same stored sera; both tissue transglutaminase and endomysial antibody tests measure the same autoantibody of sera by a different method. Still, endomysial antibody titres of the 1978–80 cohort were basically high supporting the stability of antibodies during the storage (data not shown). The stability of serum autoantibodies after long-term storage at -20 °C has also been shown in previous studies. Hence, the lower prevalence of coeliac disease in 1978–80 compared to 2000–01 is hardly likely to be because of loss of activity of antibodies during storage. Instead, if the concentration of the old stored sera had increased endomysial antibody titres, the prevalence of unrecognized coeliac disease in 1978–80 would have been overestimated in our study and the difference in the total prevalence between the two cohorts would be greater than reported.

During the study period, clinically diagnosed biopsy-proven coeliac disease cases increased many-fold. The prevalence figures for diagnosed coeliac disease of 0.03% in 1978–80 as against 0.52% in 2000–01 are fully concordant with previous Finnish prevalence studies. The rise in the prevalence of diagnosed coeliac disease is very likely due to ascertainment; a greater awareness of the disease, the increased use of serologic screening tests and good availability of open access endoscopy with routine small-bowel biopsy. Regardless of the better detection rate, 74% of coeliac disease cases still went unrecognized in 2000–01 and the finding of these cases remains a diagnostic challenge for clinicians. On the other hand, the need to diagnose all coeliac disease cases has to be proven in future studies concerning the prognosis of the disease.

In addition, we also found a statistically significantly increased prevalence of unrecognized coeliac disease (1.03% compared to 1.47%), as the 95% confidence intervals of the age- and sex-adjusted odds ratio between the study cohorts were above one. We wish to stress that the ratio of known to unrecognized coeliac disease cases varies over time and between different districts due to varying diagnostic activity. However, a changing detection rate does not influence the sum of recognized and unrecognized coeliac disease cases. Thus, if the total prevalence of coeliac disease had remained the same during the study period and diagnosed coeliac disease had increased statistically significantly as previously stated, the prevalence of unrecognized coeliac disease should have decreased instead of increasing.

The main message of the present finding is that the total prevalence of coeliac disease has increased significantly and nearly doubled during the last two decades. We carried out a novel study in coeliac disease and thus, the comparison of this result with previous studies on the same issue is impossible. However, a steady rise in the incidence of type 1 diabetes, other autoimmune diseases such as multiple sclerosis and Crohn's disease, and allergic diseases has been noted in developed countries over the last few decades. The observed rising trend in coeliac disease is parallel to that seen in type 1 diabetes in Finland (Figure 2). Such a rapid change in disease frequencies cannot be attributed to genetic changes in the population but rather to environmental factors. The reasons for such a remarkable increase in morbidity are largely unknown. According to the hygiene hypothesis the main factor underlying the increased prevalence of autoimmune diseases is the reduction in the incidence of infectious diseases. An early childhood infection or normal establishment of indigenous intestinal microbiota could down-regulate immunity and suppress different autoimmune disorders. So far, research
in the field of environmental factors affecting coeliac disease has focused on infant feeding practices. The best available evidence suggests that introducing gluten in small amounts at 4 to 6 months of age while still breastfeeding might protect from coeliac disease, but the results of the studies in question are still inconclusive.\textsuperscript{38–41} On the other hand, such changes in infant dietary practices might merely delay the clinical manifestation of coeliac disease and not inhibit the underlying process resulting in the small-intestinal coeliac lesion.\textsuperscript{38, 41} The doubled prevalence of the disorder might also be due to increased amounts of gluten in the diet after infancy.\textsuperscript{42} According to the background information (Table 1), the most significant difference between the cohorts was the improvement in educational level over time. After adjusting for educational level, the difference between the cohorts slightly decreased but remained statistically significant, indicating that the possible aetiologial factors may be both independent of and associated with education and higher socio-economic class.

As we compared two cross-sectional studies, it is necessary to discuss possible period and cohort effects. To minimize the effect of the changed diagnostic activity, we added together both diagnosed and unrecognized coeliac disease cases in calculations of the total prevalence of coeliac disease. However, it is likely that the change in the total prevalence of coeliac disease is due to some periodic or continuous environmental factors. As regards to cohort effect, we can of course not ascertain that mortality of coeliac disease population had remained the same over time. Cohort effect might partly explain our results in case that more coeliac disease cases had died before the sampling in the earlier cohort compared to the later cohort. However, a dramatic change in mortality of coeliac disease population is unlike over 20 years of follow-up and hardly explains our results.

In conclusion, the total prevalence of coeliac disease has increased considerably in Finland in the course of time. This cannot be attributed to the better detection rate and must thus reflect a true rise in the prevalence of the disorder. Identification of the environmental factors responsible for the increased frequency of coeliac disease constitutes an important issue for further studies.

**ACKNOWLEDGEMENTS**

*Authors’ declaration of personal interests:* Mr Enzo Bravi is an employee of Eurospital S.p.A., Trieste, Italy. The other authors have no conflict of interest.

*Declaration of funding interests:* The Coeliac Disease Study Group is supported by grants from the Competitive Research Funding of Pirkanmaa Hospital District, the Emil Aaltonen Foundation, the Foundation for Pediatric Research, the Yrjö Jahnsson Foundation, the Finnish Coeliac Society, the Finnish Medical Foundation and the Academy of Finland, Research Council for Health. The present study was also funded by the Commission of the European Communities in the form of the Research and Technology Development programme ‘Quality of Life and Management of Living Resources’ (QLRT-1999-00037), ‘Evaluation of the Prevalence of Coeliac Disease and its Genetic Components in the European Population’. The study does not necessarily reflect the current views or future policies of the Commission of European Communities. Authors’ work was independent of the funders.
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Malignancies in cases with screening-identified evidence of coeliac disease: a long-term population-based cohort study

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Short title: Screening-identified evidence of coeliac disease and malignancies
Key words: coeliac disease, antibodies, malignancy, complications, epidemiology
Abreviations:
Eu-tTG= a test for IgA-class tissue transglutaminase antibodies (Eu-tTG® umana IgA, Eurospital S.p.A, Trieste, Italy)
Celikey tTG = a test for IgA-class tissue transglutaminase antibodies (Celikey® Tissue Transglutaminase IgA Antibody Assay, Pharmacia Diagnostics, Uppsala, Sweden)

EMA = a test for IgA endomysial antibodies
ABSTRACT

Background and aims: The association between diagnosed coeliac disease and malignancy has been established. We studied whether previously unrecognized and thus untreated adults with screening-identified evidence of coeliac disease carry an increased risk of malignancies.

Methods: A Finnish population-based adult-representative cohort of 8000 individuals was drawn in 1978-80. Stored sera of the participants with no history of coeliac disease or any malignancy were tested for IgA-class tissue transglutaminase antibodies (Eu-tTG) in 2001. Positive sera were further analyzed by another tissue transglutaminase antibody test (Celikey tTG) and for endomysial antibodies (EMA). Malignant diseases were extracted from the nationwide database and antibody-positive were compared to negative cases during a follow-up of nearly 20 years.

Results: Altogether 565 of all the 6849 analyzed serum samples drawn in 1978-80 were Eu-tTG-positive. In further analyzes 202 (2.9 %) of the participants were Celikey tTG and 73 (1.1%) EMA-positive. The overall risk of malignancy was not increased among antibody-positive cases in the follow-up of two decades; the age- and sex-adjusted relative risk was 0.91 (95 % CI 0.60-1.37) for Celikey tTG and 0.67 (95 % CI 0.28-1.61) for EMA positives.

Conclusions: The prognosis of adults with unrecognized coeliac disease with positive coeliac disease antibody status is good as regards the overall risk of malignancies. Thus, current diagnostic practise is sufficient and there is no need for earlier diagnosis of coeliac disease by mass-screening on the basis of this study.
INTRODUCTION

Coeliac disease, which is induced by ingestion of cereal gluten, is a chronic autoimmune-mediated disease with both intestinal and extraintestinal manifestations. Screening studies have revealed up to 1-2 % seroprevalence of coeliac disease in both Europe and the USA.[1][2][3][4] However, up to 75-90 % of all cases remain unrecognized due to absent or atypical symptoms.[1][2][3][4][5][6].

According to follow-up studies, patients with diagnosed coeliac disease are at an increased risk of mortality and malignancies.[7] The risk of malignancy over all sites has been shown to be 2- to 5-fold in studies published in 1970s and 1980s.[8][9][10] Though, in recent studies the association between diagnosed coeliac disease and malignancy of any type has been much lower than previously reported, being at the most 1.3-fold.[11][12][13][14][15][16] There are solid data to suggest that long-term adherence to a gluten-free diet will reduce the incidence of complications such as malignancies.[7][10] However, a majority of diseased individuals still remain unrecognized and untreated.[5][17][18][19] We do not know whether these apparently clinically silent unrecognized cases also carry an increased risk of coeliac disease-related complications and thus, whether the health care system should recognize and treat them with a gluten-free diet during the early stages of the disease.

To elucidate this issue, we carried out a cohort study with a follow-up time of nearly 20 years and used a Finnish national population-representative adult cohort of 8000 people gathered in 1978-80. Coeliac autoantibody-positive cases in the cohort were regarded as most likely representing unrecognized coeliac disease in this study. All malignant diseases in the cohort were extracted from the Finnish cancer registry and the occurrence of any malignancies was compared between antibody-positive and -negative subjects throughout the follow-up period.
METHODS

Study population

This population-based follow-up-study took advantage of the Mini-Finland Health Survey, which was carried out in 1978-80. Details of the study design and the baseline results are extensively reported elsewhere.[20][21] In brief, a nationally representative sample of 8000 persons has been drawn of the population aged 30 and over by the stratified two-stage cluster sampling design planned by Statistics Finland. The second stage of sampling was performed in 40 areas in different parts of the country. The participants attended a health examination, which included interviews, questionnaires, drawing of blood samples and a clinical examination by a physician. The participation rate was 90 % (N=7217), and 87 % (N=6993) of the cohort had sera available for the purposes of this study in 2001 (figure 1). Cases having a previous coeliac disease or dermatitis herpetiformis diagnosis (N=3) or any malignancy (N=141) in 1978-80, when the sera were drawn and the follow-up started, were excluded from the analysis, yielding 6849 (mean age 51, range 30-95, females 3680) participants for this study (figure 1).

All participants gave informed consent. The Ethical Committee of Tampere University Hospital, Tampere, Finland, approved the study protocol.

Measurement of antibodies

The previously collected blood samples were stored at -20 C for later analysis. In 2001 all 6849 serum samples were analyzed for IgA-class tissue transglutaminase antibodies (Eu-tTG® umana IgA, Eurospital S.p.A, Trieste, Italy, abbreviated as Eu-tTG in this study). Positive sera were further analyzed using both another IgA-class tissue transglutaminase antibody kit (Celikey® Tissue Transglutaminase IgA Antibody Assay, Pharmacia Diagnostics, Uppsala,
Sweden, abbreviated as Celikey tTG), and a test for IgA endomysial antibodies (abbreviated as EMA). Both commercial tissue transglutaminase antibody kits use human recombinant tissue transglutaminase as antigen and results are given in arbitrary units (AU). The cut-off point for the Eu-tTG was 7.0 AU/ml and for the Celikey tTG 5.0 AU/ml as instructed by the manufacturer. The endomysial antibodies were defined by a standardized and validated indirect immunofluorescence method and a characteristic staining pattern at a serum dilution of 1: ≥5 was considered positive.[22][23]

Due to the unexpectedly high percentage of Eu-tTG positivity in the sera of the Mini-Finland survey collected 22 years earlier, we also randomly selected 128 (one in 50) Eu-tTG-negative serum samples and tested them for Celikey tTG and EMA. None of the them was positive for Celikey tTG or EMA (figure 1).[19]

**Malignancies and possible confounders**

Malignant diseases were identified by linking the personal identification codes with records from the nationwide database of the Finnish Cancer Registry, which includes more than 99% of incident cases diagnosed in Finland since 1953, and has been shown to be a valuable source of information as the data are of good quality with acceptably low false-positive and false-negative discrepancy rates.[24][25] Follow-up commenced the day the blood samples were drawn and the study subjects were followed up for a maximum of 19 years until the occurrence of cancer, death or the end of 1996, whichever came first, yielding a total follow-up of 103815 person years. The commonest cancers in Finland and cancers previously shown to be associated with coeliac disease were analyzed (lymphomas and breast, lung, prostate and gastrointestinal cancers).
Information on age, sex, smoking, body mass index, alcohol consumption, physical activity, bread consumption, education, number of births and menopausal status was extracted for adjustment purposes.

**Statistical analysis**

A Cox regression model (Cox 1972) was applied to estimate relative risks (RR) and 95 percent confidence intervals (95% CI) for malignancy comparing antibody-positive with antibody-negative participants. Celikey tTG-positive cases were divided into tertiles to compare relative risks at different antibody levels. The possible confounding effects of age, sex, body mass index, smoking status, alcohol consumption, physical activity, bread consumption and education were assessed using a series of multivariable models. Menopausal status and number of births were also adjusted for when analyzing the association between coeliac disease and breast cancer. We fitted multiplicative interaction terms to assess possible interactions between antibody status and age, sex, smoking and body mass index. The analyses were performed on SAS 8.02 (SAS Institute, Cary, NC).

**RESULTS**

Altogether 565 of the total 6849 analyzed serum samples were Eu-tTG-positive (figure 1). Total of 202 (2.9%, 129 females, mean age 59 years) of Eu-tTG positive cases were also Celikey tTG –positive and correspondingly 73 (1.1 %, 52 females, mean age 50 years) were EMA positive. The subjects positive for Celikey tTG were older and consumed more alcohol than negative ones. Otherwise, no statistically significant differences across antibody status were detected (table 1).

In surveillance of the participants in this study 694 (10.1 %) developed a malignancy of some type. Persons who developed cancer during the follow-up were older and were more likely to be men or smokers (data not shown).
Coeliac autoantibody positivity did not increase the overall risk of malignancy (table 2). The multivariate adjusting (age, sex, body mass index, smoking, physical activity, bread
Table 1  Association of personal characteristics with tissue transglutaminase (Celikey tTG) and endomysial (EMA) antibodies: age- and sex-adjusted distributions or mean values with standard deviations (SD) are shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Celikey tTG</th>
<th>EMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative (N=6647)</td>
<td>positive (N=202)</td>
</tr>
<tr>
<td>Men¹, %</td>
<td>46.5</td>
<td>38.6</td>
</tr>
<tr>
<td>Age², years (SD)</td>
<td>50.6 (13.9)</td>
<td>59.1  (14.2)</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SD)</td>
<td>25.9 (4.1)</td>
<td>25.6  (4.4)</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>23.9</td>
<td>19.4</td>
</tr>
<tr>
<td>Intermediate or higher education, %</td>
<td>32.6</td>
<td>31.9</td>
</tr>
<tr>
<td>Alcohol consumption, g / week (SD)</td>
<td>45.6 (106.3)</td>
<td>61.9  (150.4)</td>
</tr>
<tr>
<td>Physically active, %</td>
<td>15.4</td>
<td>13.9</td>
</tr>
<tr>
<td>Bread consumption, slices / day (SD)</td>
<td>4.8 (2.5)</td>
<td>4.9 (2.6)</td>
</tr>
<tr>
<td>Number of births³ (SD)</td>
<td>2.4 (2.0)</td>
<td>2.5 (2.7)</td>
</tr>
<tr>
<td>Postmenopausal status³, %</td>
<td>52.0</td>
<td>48.2</td>
</tr>
</tbody>
</table>
1 Age-adjusted

2 Sex-adjusted

3 Among women
**Table 2**  Age- and sex-adjusted relative risk of different cancers between persons with positive and negative tissue transglutaminase (Celikey tTG) and endomysial antibodies (EMA)

<table>
<thead>
<tr>
<th></th>
<th>Celikey tTG</th>
<th></th>
<th></th>
<th>EMA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Celikey tTG</td>
<td>P</td>
<td>EMA-</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n= 6647</td>
<td>n=202</td>
<td>n=6776</td>
<td>n=73</td>
</tr>
<tr>
<td>Cancer, all sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>671</td>
<td>23</td>
<td>689</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Relative risk (95% CI)(^1)</td>
<td>1.00</td>
<td>0.91</td>
<td>(0.60-1.37)</td>
<td>0.64</td>
<td>1.00</td>
</tr>
<tr>
<td>Lymphoproliferative disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>28</td>
<td>3</td>
<td>29</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Relative risk (95% CI)(^1)</td>
<td>1.00</td>
<td>2.76</td>
<td>(0.83-9.16)</td>
<td>0.15</td>
<td>1.00</td>
</tr>
<tr>
<td>Gastrointestinal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>115</td>
<td>6</td>
<td>121</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Relative risk and 95% CI adjusted for age and sex.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative risk (95% CI)</th>
<th>Cases</th>
<th>Relative risk (95% CI)</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>83</td>
<td>2</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.00</td>
<td>0.73 (0.18-2.97)</td>
<td>0.64</td>
<td>1.00</td>
</tr>
<tr>
<td>Breast cancer, women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases²</td>
<td>89</td>
<td>2</td>
<td>90</td>
<td>1</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.00</td>
<td>0.64 (0.16-2.59)</td>
<td>0.49</td>
<td>1.00</td>
</tr>
<tr>
<td>Prostate cancer, men</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases³</td>
<td>56</td>
<td>1</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.00</td>
<td>0.54 (0.07-3.90)</td>
<td>0.50</td>
<td>1.00</td>
</tr>
</tbody>
</table>

1 Age- and sex-adjusted

2 Only women at risk were included

3 Only men at risk were included
consumption and alcohol consumption) did not change the risk level (0.95, 95% CI 0.62-1.44, 
P=0.80) among Celikey tTG –positive cases. Nor was any association found in different levels of tissue transglutaminase antibodies or after exclusion of EMA-positive individuals from tissue transglutaminase positive cases (data not shown).

However, EMA positivity was statistically significantly associated with an increased risk of lymphoproliferative disease (table 2). When non-Hodgkin’s lymphomas were considered separately, the age- and sex-adjusted relative risk of this malignancy among EMA-positive cases was 6.43 (95 % CI 1.52-27.22, p=0.05, N=2) and the corresponding risk for Celikey tTG-positive individuals was 2.92 (95 % CI 0.87-9.74, p=0.13, N=3). The duration between known antibody positivity and the diagnosis of non-Hodgkin lymphoma varied between 6-14 years and non-Hodgkin lymphoma had no specific site predilection (table 3). There were no enteropathy-associated T-cell lymphomas. In addition, the age- and sex-adjusted relative risk of carcinoma of the oesophagus was increased among Celikey tTG-positive cases being 7.48 (95% CI 2.06-27.25, p=0.01, N=3).

We also adjusted the relative risks for non-Hodgkin’s lymphoma and carcinoma of the oesophagus for alcohol consumption, as this differed between antibody-positive and -negative participants (table 1); the risks remained virtually the same after the adjustment. No statistically significant interactions between any of the potentially effect-modifying factors (age, sex, smoking and body mass index) and antibody status were noted in the prediction of malignancy of any type (data not shown).

DISCUSSION

Several studies have investigated the association between diagnosed and treated coeliac disease and malignancies. [8][9][10][11][12][13][14][15][16] We still do not know whether
Table 3. Characteristics of non-Hodgkin’s lymphoma (NHL) and carcinoma of the oesophagus in coeliac autoantibody-positive cases

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age at the beginning of the follow-up</th>
<th>Age at the time of cancer diagnosis</th>
<th>Organ involved; histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>58</td>
<td>72</td>
<td>groin and low extremities; NHL</td>
</tr>
<tr>
<td>Female</td>
<td>63</td>
<td>74</td>
<td>tonsils; NHL</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>62</td>
<td>skin; NHL</td>
</tr>
<tr>
<td>Female</td>
<td>63</td>
<td>80</td>
<td>oesophagus; squamous cell carcinoma</td>
</tr>
<tr>
<td>Male</td>
<td>59</td>
<td>76</td>
<td>oesophagus; squamous cell carcinoma</td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>84</td>
<td>oesophagus; adenocarcinoma</td>
</tr>
</tbody>
</table>
apparently asymptomatic unrecognized coeliac disease cases are at an increased risk of cancers. We now carried out a cohort study of the association of coeliac autoantibodies and malignancies with a follow-up time of nearly twenty years. The majority of previously unrecognized antibody-positive cases would most likely have remained undiagnosed throughout the follow-up period, as according to our previous data from Finland, 74 % of coeliac disease cases still remained unrecognized in 2000.[19] Our study design enabled us to approach a hitherto unexplored issue, the prognosis of the unrecognized part of the coeliac population with regard to any cancers.

The present study showed that there is no additional risk of overall malignancy among untreated adults with screening-identified evidence of coeliac disease in a follow-up of nearly twenty years. In the majority of recently published studies the association between diagnosed coeliac disease and malignancy of any type has likewise not been found.[11][12][13][14][15][16] In contrast, the risk of malignancy over all sites was increased in studies published in the 1970s and 1980s.[8][9][10] The decrease in the risk of malignancy during the last few decades may be due to improved diagnostic activity, the increased number of coeliac disease cases with mild symptoms over time and thus, early commitment to a gluten-free diet.[10] As also discussed by Catassi and associates,[26] a detection bias might also have caused overestimation of the risk of malignancy in the earliest studies. The likelihood of detecting an occult or overt malignancy in individuals with coeliac disease may be higher compared to the corresponding likelihood in a control group due to more careful examination of the diseased cases. There are no previous follow-up studies comparable to ours concerning the association of unrecognized coeliac disease with malignancies. However, the same issue has been approached from a different point of view with inconclusive results. Metzger and colleagues studied mortality among a tissue transglutaminase antibody-positive population and found a 3.6-fold excess of cancer as cause of death during eight years of
In contrast, no increased risk of non-Hodgkin’s lymphoma could be detected in a small subgroup of screen-detected silent coeliac disease cases in a case-control study design. We studied previously unrecognized coeliac autoantibody-positive cases and as they most probably had a mild clinical picture, the risk of complications such as malignancies seemed to remain low.

However, earlier unrecognized coeliac autoantibody-positive cases may still carry an increased risk of specific malignancies such as non-Hodgkin’s lymphoma and carcinoma of the oesophagus. The number of detected malignancies remained low regardless of the follow-up time of nearly 20 years, and thus caution is warranted in interpretation of the results. However, our results are supported by earlier findings whereby diagnosed coeliac disease patients have repeatedly been at an increased risk of the same specific cancers.

Since 2002 all coeliac disease or dermatitis herpetiformis patients in Finland have had a right to financial assistance from the Social Insurance Institution provided the diagnostic criteria for the diseases are fulfilled. According to the register only four coeliac autoantibody-positive cases had received a correct diagnosis by the end of the follow-up period and only two of them followed a gluten-free diet (unpublished data). As only patients alive in 2002 were included in the register, there may be few diagnosed cases we could not detect. Nonetheless, the number of diagnosed and treated cases during the follow-up seems to be low and thus, the major part of the unrecognized coeliac autoantibody-positive cases in 1978-80 had also remained unrecognized and untreated throughout the follow-up period.

According to the literature, IgA-class tissue transglutaminase and endomysial antibodies are valid tests for coeliac disease. The pooled specificities of these antibodies have been reported to approach 100% and sensitivities to be mainly over 90% in adult populations. As a result of imperfect sensitivity there may be some false negative
coeliac disease cases in our cohort due to either IgA deficiency or totally lacking coeliac autoantibodies thus possibly slightly diluting the real difference. However, these autoantibodies have previously been used in large screening studies in the USA and Europe[1][3][4] and some centres even use serology as a diagnostic tool without intestinal biopsy.[31] The detection of a surprisingly high frequency of Eu-tTG positivity possibly due to long storage has earlier been discussed.[19] For the clarity and the low specificity of the test we do not report the results based on that test though the results were parallel with reported results with other antibody tests (data not shown). The problem with old sera could theoretically be fully obviated by a prospective study design, which would however take decades to yield information. Additionally, it might be unethical to follow cases with apparent coeliac disease for several years without treatment. Nonetheless, the validity of our conclusions was strengthened as we obtained parallel results independent of the antibody used.

In conclusion, the overall risk of malignancies was not increased among coeliac autoantibody-positive cases probably representing unrecognized coeliac disease. Thus early diagnosis of coeliac disease through serological mass screening would not be beneficial in improving the prognosis of these antibody-positive cases as regards malignancies.
COMPETING INTERESTS

The authors have nothing to disclose.
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REFERENCES


FIGURE LEGENDS

**Figure 1** Flow-chart of the present study. Eu-tTG= IgA-class tissue transglutaminase antibody (Eu-tTG® umana IgA), Celikey tTG= IgA-class tissue transglutaminase antibody (Celikey® Tissue Transglutaminase IgA Antibody Assay), EMA= IgA-class endomysial antibody, (*)= Cases with any malignancy (N=141) or previous coeliac disease or dermatitis herpetiformis diagnosis (N=3)
ORIGINAL ARTICLE

Prognosis of unrecognized coeliac disease as regards mortality: A population-based cohort study

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Abstract

Background and aim. Clinically diagnosed coeliac disease patients carry an increased risk of mortality. As coeliac disease is markedly underdiagnosed, we aimed to quantify the risk of mortality in subjects with unrecognized and thus untreated coeliac disease.

Method. Blood samples from 6,987 Finnish adults were drawn in 1978–80, and sera were tested for immunoglobulin A (IgA)-class tissue transglutaminase antibodies (Eu-tTG) in 2001. Positive sera were further analysed for endomysial (EMA) and tissue transglutaminase antibodies by another test (Celikey tTG). EMA- and Celikey tTG-positive cases were compared to negatives as regards mortality in up to 28 years of surveillance, yielding a total follow-up of 147,646 person years. Dates and causes of death were extracted from the nation-wide database.

Results. Altogether 74 (1.1%) of the participants were EMA- and 204 (2.9%) Celikey tTG-positive. The age- and sex-adjusted relative risk of overall mortality was not increased in either EMA (0.78, 95% CI 0.52–1.18) or Celikey tTG (1.19, 95% CI 0.99–1.42)-positive subjects. However, antibody-positive cases evinced a tendency to die from lymphoma, stroke, and diseases of the respiratory system.

Conclusions. The prognosis of unrecognized coeliac disease was good as regards overall mortality, which does not support screening of asymptomatic coeliac disease cases.

Key words: Antibody, coeliac disease, epidemiology, mortality

Introduction

Coeliac disease is a chronic autoimmune-like disorder with intestinal and extraintestinal manifestations induced by wheat gluten and related proteins of rye and barley. Conceptions of the epidemiology of coeliac disease have changed substantially over time. It is currently considered a global prevalent disorder increasing over time and affecting up to 1%–2% of the Western population (1–5). Diarrhoea and malabsorption as clinical manifestations are nowadays more rarely seen, and up to 70%–90% of the coeliac disease population in Western countries remain unrecognized due to the absence or the atypical nature of symptoms (1–5). Prognostic studies on undiagnosed and thus untreated cases are scant, and debate thus continues as to whether health care professionals should rigorously seek out these cases even by population mass-screening (6–11).

Diagnosed clinically detected coeliac disease cases carry a 1.3–3.8-fold increased risk of mortality mainly attributable to malignancies, and the risk seems to decrease on a gluten-free diet (12–20). However, there have been only two previous attempts to assess the association between unrecognized coeliac disease and mortality (21,22). Due to
the relatively short follow-up times and modest number of antibody-positive cases in the studies in question, the results remain inconclusive.

We followed a large Finnish population-based adult-representative cohort of 6,987 people collected in 1978–80, focusing on mortality and aiming to establish whether unrecognized coeliac disease individuals carry an increased risk of mortality during a surveillance of up to 28 years. Sequential analysis of sera from the participants by tissue transglutaminase and endomysial antibody tests with reported high validity (23,24) enabled us to define coeliac autoantibody-positive cases most likely representing unrecognized coeliac disease. We availed ourselves of the causes of death register of Statistics Finland and could compare the overall and cause-specific mortality between antibody-positive and -negative subjects throughout the follow-up period.

**Key messages**

- The prognosis of undetected coeliac disease is good as regards overall mortality.
- Coeliac antibody-positive undetected cases evinced an additional tendency to die from lymphoma, stroke, and diseases of the respiratory system.

**Material and methods**

**Study population**

The Mini-Finland Health Survey carried out in 1978–80 provided a basis for the current population-based follow-up study between autoantibody-positive unrecognized coeliac disease and mortality. Detailed information on the design and base-line results of the primary study has been published elsewhere (25,26). In brief, a nationally representative sample of 8,000 persons was drawn from the population aged 30–99 years according to a stratified two-stage cluster-sampling model planned by Statistics Finland. The second stage of sampling was implemented in 40 areas in different parts of the country. The participants attended a health examination which included interviews, questionnaires, drawing of blood samples, and a clinical examination by a physician.

The participation rate was 90% (n = 7,217), and sera from 6,990 individuals were still available for the purposes of this study in 2001 (Figure 1). Three coeliac disease or dermatitis herpetiformis cases previously diagnosed and treated were excluded from the analysis at the beginning of the follow-up, yielding a total of 6,987 participants (3,766 females, mean age 51 years, age range 30–95) for this study (Figure 1). Information on age, sex, education, body mass index, alcohol consumption, smoking, hypertension, serum cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, diabetes, coronary heart disease, stroke, and cancer were extracted for purposes of adjustment. The definitions and measurement of potential confounding and effect-modifying factors have been described in greater detail elsewhere (25,26). All participants gave informed consent. The Ethical Committee of Tampere University Hospital approved the study protocol.

**Measurement of antibodies**

Serum samples from the participants were stored at −20°C, and altogether 6,987 sera were analysed for immunoglobulin A (IgA)-class tissue transglutaminase antibodies in the first stage of screening (Eu-tTG <xref ref-type="fn" rid="fn28">†</xref>umana IgA, Eurospital S.p.A., Trieste, Italy; abbreviated as Eu-tTG) in 2001 (Figure 1). Further, positive sera were analysed parallelly for both IgA endomysial antibodies (abbreviated as EMA) and using another IgA-class tissue transglutaminase antibody kit (Celikey<sup>®</sup>, Phadia, Freiburg, Germany; abbreviated as Celikey tTG) (Figure 1). The endomysial antibodies were defined by a standardized and validated indirect immunofluorescence method using human umbilical cord as antigen, and a characteristic staining pattern at a serum dilution 1: ≥5 was considered positive (27,28). Both commercial tissue transglutaminase antibody kits use human recombinant tissue transglutaminase as antigen, and results are given in arbitrary units (AU). The cut-off point for the Eu-tTG was 7.0 AU/mL and for the Celikey tTG 5.0 AU/mL according to manufacturers’ instructions. These IgA-class tissue transglutaminase and EMA tests have been shown to be valid for coeliac disease, with pooled specificities approaching 100% and sensitivities mainly over 90% in adult populations (24,29). In accordance with previous studies (5,30)

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celikey</td>
<td>a test for immunoglobulin A (IgA)-class antibodies (Celikey&lt;sup&gt;®&lt;/sup&gt;, Phadia, Freiburg, Germany)</td>
</tr>
<tr>
<td>EMA</td>
<td>a test for IgA endomysial antibodies</td>
</tr>
<tr>
<td>Eu-tTG</td>
<td>a test for IgA-class tissue transglutaminase antibodies (Eu-tTG&lt;sup&gt;®&lt;/sup&gt; umana IgA, Eurospital S.p.A., Trieste, Italy)</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IgA</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>tTG</td>
<td>tissue transglutaminase antibodies</td>
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</tbody>
</table>
the definition of unrecognized coeliac disease was based on a two-stage screening algorithm where cases yielding a positive result for both Eu-tTG and either EMA or Celikey tTG were considered to constitute undetected coeliac disease. The main motivation for the two-stage screening algorithm was to decrease the likelihood of false-positive results and thus the dilution of a real effect. Furthermore, to find a close estimate of the real risk we defined unrecognized coeliac disease by two different antibody tests (Celikey tTG and EMA) in the second stage of screening.

In view of the unexpectedly high number of Eu-tTG-positive sera collected 22 years earlier, we wished to check that the influence of storage on Eu-tTG values was not arbitrary. We therefore also tested 128 (1 in 50) randomly selected Eu-tTG-negative sera for EMA and Celikey tTG (Figure 1). As none were positive, we could ascertain that the likelihood of coeliac disease in Eu-tTG-negative individuals was low (Figure 1). We have previously extensively discussed the possible influence of storage of sera on coeliac autoantibodies (5,30).

**Mortality**

Date and cause of death were identified by linking the unique personal identification codes with records from the nation-wide database of Statistics Finland (31). The principal causes of death were coded either according to International Classification of Diseases (ICD)-8, -9, or -10, depending on the time of death. Follow-up commenced the day the blood samples were drawn in 1978/80, and the study subjects were under surveillance until the end of 2005 or death; this yielded a total follow-up of 147,646 person years. The maximum follow-up time was 28 years. The mortality among antibody-positive cases was compared to that of antibody-negative individuals in the same cohort.

**Statistical analysis**

Adjusted relative risks (RR) and their 95% confidence intervals (95% CI) for mortality comparing antibody-positive with antibody-negative participants were estimated based on a Cox regression model (Cox 1972). Statistical significance of heterogeneity was tested using the likelihood ratio test based on the model and expressed by P-value. The possible confounding effects of age, sex, education, body mass index, alcohol consumption, smoking, hypertension, serum cholesterol, high-density lipoprotein and triglycerides, diabetes, coronary heart disease, stroke, and cancer were assessed using a
series of multivariate models. Stratified analyses were conducted to assess mortality risk also according to the level of antibodies (titres 1: <500 and 1: \( \geq 500 \) in EMA, and <6.4 and \( \geq 6.4 \) AU/mL in Celikey tTG, divided by medians of positive values) and length of follow-up (1–10 years and >10 years). In addition, potential effect-modifying factors such as age, sex, education, body mass index, alcohol consumption, smoking, hypertension, and cholesterol values were entered into the models. The analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA).

Results

Altogether 574 out of the 6,987 analysed serum samples proved Eu-tTG-positive (Figure 1). Seventy-four (53 females, mean age 49 years) out of the Eu-tTG-positive had positive EMA, and 204 (125 females, mean age 59 years) positive Celikey tTG. Thus, 1.1% of the 6,987 analysed samples were EMA-positive and, correspondingly, 2.9% Celikey tTG-positive.

As to the personal characteristics of the screened population, subjects positive in either EMA or Celikey tTG had better cholesterol profiles compared to antibody-negative participants (Table I). In addition, Celikey tTG-positive cases were older, consumed more alcohol, and more likely suffered from diabetes than antibody-negative subjects. Otherwise, no statistically significant differences were detected across antibody status. A total of 3,069 (43.9%) participants out of the 6,987 died during the surveillance.

No increased age- and sex-adjusted relative risk of overall mortality could be detected among EMA-positive cases, but there was border-line significant modestly elevated risk of overall mortality in Celikey tTG-positive individuals (Table II). The risk levels remained virtually the same after further adjustment for body mass index, smoking, education, alcohol consumption, hypertension, serum cholesterol profile, diabetes, coronary disease, stroke, and cancer. Nor was the risk statistically significantly different in the first 10 years of follow-up (EMA-positive 0.36 (95% CI 0.12–1.11, \( P=0.03 \); Celikey tTG-positive 1.17 (95% CI 0.89–1.54, \( P=0.26 \)) or thereafter (EMA-positive 0.95 (95% CI 0.61–1.47, \( P=0.81 \); Celikey tTG-positive 1.23 (95% CI 0.98–1.56, \( P=0.09 \)). Furthermore, high EMA titre had likewise no influence on risk level (0.74, 95% CI 0.42–1.30, \( P=0.45 \)), but individuals with high Celikey tTG levels had a border-line significantly elevated risk of overall mortality (1.32, 95% CI 1.00–1.72, \( P=0.12 \)). No statistically significant interactions between any of the potentially effect-modifying factors and antibody status were noted in the prediction of all-cause mortality (data not shown).

Diseases of the circulatory system and malignant neoplasm were leading causes of death among both the antibody-positive and -negative population, comprising 72.0% of all deaths. Antibody-positive

Table I. Association of personal characteristics with endomysial (EMA) and tissue transglutaminase (Celikey tTG) antibodies: age- and sex-adjusted distributions or mean values with standard deviations (SD) are shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EMA Negative (n = 6913)</th>
<th>EMA Positive (n = 74)</th>
<th>P-value</th>
<th>Celikey tTG Negative (n = 6783)</th>
<th>Celikey tTG Positive (n = 204)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men*, %</td>
<td>46.3</td>
<td>28.0</td>
<td>0.002</td>
<td>46.3</td>
<td>38.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Ageb, years (SD)</td>
<td>51.1 (14.1)</td>
<td>49.2 (11.8)</td>
<td>0.26</td>
<td>50.8 (14.0)</td>
<td>59.1 (14.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intermediate or higher education, %</td>
<td>32.4</td>
<td>36.1</td>
<td>0.47</td>
<td>32.3</td>
<td>32.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Body mass index, kg/m² (SD)</td>
<td>25.9 (4.1)</td>
<td>25.0 (3.9)</td>
<td>0.07</td>
<td>25.9 (4.1)</td>
<td>25.6 (4.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>23.7</td>
<td>19.6</td>
<td>0.38</td>
<td>23.8</td>
<td>19.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Physically active, %</td>
<td>15.3</td>
<td>15.1</td>
<td>0.96</td>
<td>15.3</td>
<td>13.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Alcohol consumption, g/week (SD)</td>
<td>45.9 (107.8)</td>
<td>33.8 (80.8)</td>
<td>0.31</td>
<td>45.3 (106.1)</td>
<td>60.8 (149.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hypertensive, %</td>
<td>22.7</td>
<td>20.2</td>
<td>0.60</td>
<td>22.6</td>
<td>26.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (SD)</td>
<td>6.95 (1.37)</td>
<td>6.36 (1.16)</td>
<td>&lt;0.001</td>
<td>6.96 (1.36)</td>
<td>6.37 (1.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL, mmol/L (SD)</td>
<td>1.70 (0.41)</td>
<td>1.50 (0.32)</td>
<td>&lt;0.001</td>
<td>1.70 (0.41)</td>
<td>1.55 (0.38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L (SD)</td>
<td>1.54 (1.08)</td>
<td>1.38 (0.49)</td>
<td>0.19</td>
<td>1.53 (1.08)</td>
<td>1.50 (0.73)</td>
<td>0.67</td>
</tr>
<tr>
<td>LDL, mmol/L (SD)</td>
<td>4.56 (1.24)</td>
<td>4.24 (1.04)</td>
<td>0.02</td>
<td>4.57 (1.24)</td>
<td>4.15 (1.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>5.7</td>
<td>3.3</td>
<td>0.35</td>
<td>5.5</td>
<td>11.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary disease, %</td>
<td>10.9</td>
<td>5.8</td>
<td>0.14</td>
<td>10.9</td>
<td>10.0</td>
<td>0.66</td>
</tr>
<tr>
<td>Stroke, %</td>
<td>1.6</td>
<td>1.7</td>
<td>0.95</td>
<td>1.5</td>
<td>3.1</td>
<td>0.08</td>
</tr>
<tr>
<td>Cancer, %</td>
<td>2.2</td>
<td>1.4</td>
<td>0.64</td>
<td>2.2</td>
<td>0.1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*aAge-adjusted.

*bSex-adjusted.

HDL = serum high-density lipoprotein; LDL = serum low-density lipoprotein.
cases ran an increased risk of death from lymphoma (Table III). Equally, the risk estimates for stroke and diseases of the respiratory system were increased in both EMA- and Celikey tTG-positive subjects. The risk estimate for dementia as a cause of death was increased only in Celikey tTG-positive cases (Table III).

Discussion

No statistically significantly increased risk of all-cause mortality was detected among coeliac antibody-positive unrecognized coeliac disease cases. This is in contrast to findings in earlier studies in apparently symptomatic clinically detected coeliac disease patients, which have shown an increased risk of overall mortality (12–20). The association between unrecognized coeliac disease and mortality has previously been approached in only two separate studies, with discrepant results (21,22). Metzger and associates could show a 2.5-fold increased risk of overall mortality among tissue transglutaminase antibody-positive cases (22). Their cases were mostly men, whereas in coeliac disease in general females predominate (32). Comparable to our findings again, Johnston and colleagues (21) found no excess risk, but their cohort consisted of only few EMA- or antireticulin antibody-positive cases. However, it remains unclear whether a border-line statistically significant 19% increased risk of overall mortality among Celikey tTG-positive cases of our study could reach statistical significance in still larger settings. We hypothesize that any possible difference in risk of mortality between endomysial and tissue transglutaminase antibody-positive individuals might be due to border-line positive tissue transglutaminase antibodies outside celiac disease or reflect different subtypes of coeliac disease.

Relating to cause-specific mortality, malignancies at any site were not in general overrepresented in unrecognized coeliac disease. However, albeit based on few cases, the present study indicated that non-Hodgkin’s lymphoma as cause of death was overrepresented in undetected coeliac disease. The association is strongly supported by the previous literature, where a connection between diagnosed coeliac disease and non-Hodgkin’s lymphoma has repeatedly been reported (15,16,18,20,33). As to diseases of the circulatory system, the association with coeliac disease has remained inconclusive (16,18,20,34,35). We could show lower cholesterol levels in antibody-positive compared to antibody-negative individuals. Even though the mechanisms for the phenomenon should be evaluated in further studies, we suggest that it might be due to impaired absorption in the intestine. A favourable cardiovascular risk profile has also previously been connected with undetected coeliac disease (3), thus possibly reducing the risk of diseases of the circulatory system. On the other hand, malabsorption of folic acid followed by hyperhomocysteinaemia might increase the risk of these diseases (36–38) and explain the increased risk of stroke in unrecognized coeliac disease cases in the current study. Furthermore, an excess risk of diseases of the respiratory system in general could be demonstrated in our study, as has previously been reported in diagnosed cases (15,16). According to the previous literature, specific illnesses such as tuberculosis (39), other lung cavities (40), and sarcoidosis (41) might be in the background.

We are confident regarding the main results of the present study, as we used an adult-representative population-based cohort with a high participation rate (90%) and a substantial number (147,646) of person years, and further tested all sera from the
Table III. Age- and sex-adjusted relative risks of cause-specific mortality between persons with positive and negative endomysial (EMA) and tissue transglutaminase (Celikey tTG) antibodies.

<table>
<thead>
<tr>
<th>Cause of death (ICD code)</th>
<th>EMA</th>
<th></th>
<th>Celitkey tTG</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>RR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Malignant neoplasms (C00-C97)</td>
<td>655</td>
<td>5</td>
<td>0.73 (0.30-1.77)</td>
<td>0.47</td>
</tr>
<tr>
<td>Digestive organs (C15-C26)</td>
<td>234</td>
<td>1</td>
<td>0.41 (0.06-2.89)</td>
<td>0.29</td>
</tr>
<tr>
<td>Lymphoma (C81-C88)</td>
<td>20</td>
<td>2</td>
<td>9.51 (2.20-41.22)</td>
<td>0.02f</td>
</tr>
<tr>
<td>Diseases of the circulatory system (I00-I99)</td>
<td>1539</td>
<td>12</td>
<td>0.85 (0.48-1.50)</td>
<td>0.57</td>
</tr>
<tr>
<td>Ischemic heart diseases (I20-I25)</td>
<td>930</td>
<td>4</td>
<td>0.49 (0.19-1.30)</td>
<td>0.10</td>
</tr>
<tr>
<td>Stroke (I60-I69)</td>
<td>354</td>
<td>4</td>
<td>1.20 (0.45-3.23)</td>
<td>0.72</td>
</tr>
<tr>
<td>Diseases of the digestive system (K00-K93)</td>
<td>94</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endocrine diseases (E00-E35)</td>
<td>42</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diseases of the respiratory system (J00-J99)</td>
<td>260</td>
<td>3</td>
<td>1.47 (0.47-4.61)</td>
<td>0.53</td>
</tr>
<tr>
<td>Dementia (F00-F03, G30, R54)</td>
<td>152</td>
<td>1</td>
<td>0.70 (0.10-5.04)</td>
<td>0.71</td>
</tr>
<tr>
<td>Accidents, suicide and violence (S00-S99; T00-T98; V01-V99; W00-W99; X00-X99; Y00-Y98)</td>
<td>160</td>
<td>1</td>
<td>0.63 (0.09-4.50)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

aDue to the low number (0-1) of cases with diseases of the nervous system, infectious and parasitic conditions, musculoskeletal system and connective tissue diseases or specific malignancies as cause of death in the antibody-positive group, the results are not shown.
bRelevant International Classification of Diseases codes (ICD-8, -9, and -10) were used. Corresponding ICD-10 codes are shown.
cRelative risk estimated by a Cox regression model.
dThe analysis was repeated after exclusion of cases with the mentioned illness at the beginning of follow-up, and the results remained virtually the same.
eExclusion of cases with the defined illness at the beginning of follow-up was not possible.
fP-value < 0.05.
participants by a two-stage screening-algorithm. In addition, with a cohort study design with historical components we could avoid the possible ethical concern associated with a fully prospective design, i.e. following up diagnosed coeliac disease cases without treatment for decades. Nor would a fully prospective study have yielded results in reasonable time. By our population-based screening we were able to avoid confining participants to the most serious cases as is commonly the case in studies with clinically diagnosed coeliac disease. In addition, we compared antibody-positive and -negative individuals of the same cohort and could thus adjust for several potential confounding factors (42). However, imperfect sensitivity due to IgA deficiency could slightly dilute the real effect (43). As to the outcome variable, the causes of death register has included all deaths in Finland since 1936, and good validity and coverage of the data in the register have been reported (31,44). In brief, details of our study design strengthen the validity of the present results on the prognosis of the unrecognized section of the coeliac population.

Since it might be asked whether good prognosis as regards overall mortality is due to the diagnosis and treatment of unrecognized cases during surveillance, we evaluated risk estimates in the first 10 years of follow-up and thereafter. We found no increased risk of mortality in the first period of follow-up, where the likelihood of coeliac disease diagnosis most probably remained low, or later on. However, individuals with higher levels of tissue transglutaminase antibodies carried a modestly increased risk of mortality, this possibly being explained by more severe disease in these cases (15,45–47).

The good prognosis found here as regards overall mortality would suggest that a search for asymptomatic coeliac disease by screening programmes is not warranted even though the mortality risks of specific diseases were increased. Other outcome variables such as fractures and quality of life in undetected disease should still be evaluated in future studies to obtain an overall picture of the entity of undetected coeliac disease.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


