KIRSI MUSTALAHTI

Silent Coeliac Disease

ACADEMIC DISSERTATION
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”When one door of happiness closes, another opens.”

Helen Keller

To my children

ABSTRACT

Silent coeliac disease can be definied as a disorder in which an individual has the typical coeliac disease-specific small-bowel mucosal lesion but clinical symptoms are lacking. The background to the varying clinical pictures of the disease is unknown. Silent coeliac disease remains undiagnosed without active serological screening. The benefits of gluten-free diet treatment in silent coeliac disease have as yet been little investigated.

In this study the prevalence of coeliac disease was studied among an unselected population of 3654 schoolchildren and 466 first-degree relatives of coeliac disease patients. The screening methods used were endomysial antibodies as measured by indirect immunofluorescense, and tissue transglutaminase antibodies measured with commercial kits based on the enzyme-linked immunosorbent assay (ELISA) method. Coeliac disease diagnoses were confirmed by small-bowel biopsies whenever the consent of the patient could be obtained. Human leucocyte antigen (HLA)-DQ2 and –DQ8 were determined in all schoolchildren and all newly diagnosed coeliac disease patients among first-degree relatives of coeliac disease patients, and in a group of previously diagnosed coeliac disease patients. The antibody results were correlated to each other and to coeliac disease related HLA haplotypes.

In order to establish the possible role of HLA DR-DQ haplotypes in discriminating between the clinically silent and symptomatic forms of coeliac disease, HLA DR-DQ distribution was evaluated between 28 siblings with different forms of the disease. DQB1 typing was based on polymerase chain reaction with allele-specific primers identifying the major DQ allele groups (DQ2-DQ9) and was carried out with a Dynal DQ "low resolution" SSP kit.

The benefit of gluten-free diet treatment in silent coeliac disease was assessed by measuring the bone mineral density of the spine and femoral neck of 19 silent coeliac disease patients by dual-energy X-ray absorptiometry at time of diagnosis and after one year on a diet. Thirty symptomatic patients served as controls and the baseline bone mineral density data were expressed as t-scores with reference to sex-matched young normal data.

Further, changes in the quality of life in 19 silent coeliac disease patients and 21 symptomatic patients were evaluated on a gluten-free diet. Two self-administered questionnaires were used: psychological general well-being (PGWB), and gastrointestinal rating scale (GSRS).

Among an unselected sample of schoolchildren 10 coeliac disease cases were diagnosed because of clinical symptoms and 27 biopsy-proven new cases were diagnosed on the basis of serological screening, giving a biopsy-proven prevalence of 1:99. There were altogether 55 individuals in this material

fulfilling the criteria for genetic gluten intolerance (autoantibody positivity and coeliac disease associated HLA haplotype), giving a prevalence figure as high as 1:67. One third of the cases were symptomless.

Among 466 first-degree relatives of multiple case families, despite a high index of suspicion of the disease, there were still altogether 44 endomysial antibody-positive individuals. All of them carried the coeliac disease associated HLA haplotypes. Twenty-eight out of 31 biopsied subjects showed small-bowel mucosal lesion typical of coeliac disease.

The endomysial and tissue transglutaminase antibody results correlated well with each other and with high-risk HLA haplotypes associated with coeliac disease. The most potent risk factor for coeliac disease was HLA DR3-DQ2 (Odds ratio 26.45). There were no statistically significant differences in HLA DR-DQ distributions between the siblings with silent coeliac disease and those with the symptomatic form. Homozygous individuals were not overrepresented in the group of symptomatic patients.

Bone mineral density measurements showed that at the time of diagnosis the median t-scores of silent coeliac disease patients were decreased in both spine (-1.9, 95% CI -2.4 to -1.4) and femoral neck -0.9 (95% CI -1.5 to -0.7). After one year on a gluten-free diet the spinal bone mineral density was significantly increased in eight and the femoral neck bone mineral density in ten of 19 silent coeliac disease patients.

At diagnosis the quality of life scores of silent coeliac disease patients did not diverse from those of non-coeliac controls but in symptomatic patients quality of life of was significantly impaired ($p<0.01$) when compared to the other groups. After 1 year on a gluten-free diet, mean PGWB scores increased from 108 (95% CI 103 to 113) to 114 (95% CI 110 to 118) among silent coeliac disease patients and from 92 (95% CI 85 to 99) to 103 (95% CI 97 to 109) in symptomatic patients.

In conclusion, it is obvious that silent coeliac disease is common in the Finnish population: in about one third of screen-detected coeliac disease patients clinical symptoms are lacking. The true prevalence of coeliac disease or genetic gluten intolerance is thus even higher than previously believed. The genetic or environmental factors affecting the clinical picture of the disease remain to be identified. In the case of bone mineral density, however, gluten-free diet treatment would appear to be beneficial for both symptomatic and silent coeliac disease patients and the treatment does not atleast worsen the quality of life. However, many questions remain still to be solved before any decision can be made as to whether silent coeliac disease should be commonly screened in the population. The benefits of gluten-free diet treatment should be thoroughly evaluated in the context of all the complications known to be associated with coeliac disease, and cost-benefit calculations should be made before population-based screening can be proposed or rejected. Meanwhile, screening for coeliac disease among risk groups is to be recommended: provided the disease is borne in mind it is easy to find.

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ABBREVIATIONS

AGA	gliadin antibody
ANOVA	analysis of variance
ARA	reticulin antibody
AU	arbitrary unit
BMD	bone mineral density
CI	confidence interval
ELISA	enzyme-linked immunosorbent assay
EMA	endomysial antibody
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
GFD	gluten-free diet
GSRS	gastrointestinal symptoms rating scale
HLA	human leukocyte antigen
IgA	immunoglobulin A
IEL	intraepithelial lymphocyte
OR	Odds ratio
PGWB	psychological general well-being
PPV	positive predictive value
TcR	T-cell receptor
tTG	tissue transglutaminase

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original communications, referred to in the text by Roman numerals I-V.

I Mäki M., Mustalahti K., Kokkonen J., Kulmala P., Haapalahti M., Karttunen T., Ilonen J., Laurila K., Dahlbom I., Hansson T., Höpfl P., Knip M.: Prevalence of celiac disease among children in Finland. New England J Med 2003;348:2517-24. (Reprinted with permission from Massachusetts Medical Society)

II Mustalahti K., Sulkanen S., Holopainen P., Laurila K., Collin P., Partanen J., Mäki M.: Coeliac disease among healthy members of multiple case coeliac disease families. Scand J Gastroenterol 2002;37:161-65. (Reprinted with permission from Taylor & Francis AS)

III Mustalahti K., Holopainen P., Karell K., Mäki M., Partanen J.: Genetic dissection between the silent and clinically diagnosed symptomatic forms of coeliac disease in multiplex families. Dig Liver Dis 2002;34;842-5. (Reprinted with permission from Dig Liver Dis)

IV Mustalahti K., Collin P., Sievänen H., Salmi J., Mäki M.: Osteopenia in patients with clinically silent coeliac disease warrants screening. Lancet 1999;354:744-45. (Reprinted with permission from Elsevier Science)

V Mustalahti K., Lohiniemi S., Collin P., Vuolteenaho N., Laippala P., Mäki M.: Gluten-free diet and quality of life in patients with screen-detected celiac disease. Eff Clin Pract 2002;5:105-13. (Reprinted with permission from Ann Int Med)

INTRODUCTION

Coeliac disease is an autoimmune-type disorder which in genetically susceptible individuals is caused by daily ingested gluten - the protein fraction in wheat, rye and barley. In its classical form coeliac disease is characterized by severe gastrointestinal symptoms and malabsorption, and in children failure to thrive. The diagnosis is based on disease-specific autoantibodies and small-bowel mucosal findings of villous atrophy, crypt hyperplasia and an increased number of intraepithelial lymphocytes.

It is now known that the clinical picture of coeliac disease is much more complicated than previously thought. The condition may be totally symptomless despite severe mucosal damage, or it may cause only extraintestinal manifestations such as the bullous itching excema called dermatitis herpetiformis, short stature in children of isolated iron deficiency anaemia. Irrespective of the symptoms the small-bowel mucosal findings may vary from mild inflammation of the mucosa to extensive damage of the villi and severe hyperplasia of the crypts (Mäki and Collin 1997).

Since the clinical picture of the disease is complicated, its true prevalence is difficult to estimate. Classically the prevalence was estimated to be 1:1000, this figure including clinically diagnosed individuals with classical gastrointestinal symptoms. If individuals screened for coeliac disease because of mild or atypical symptoms as well as individuals belonging to risk groups are taken into account, the prevalence of the disease is 1:300 (Collin et al. 1995). This figure may likewise be underestimated since symptomless patients with a manifest mucosal lesion remain undetected without active population-based screening.

Currently we have efficient tools for the screening of coeliac disease. Endomysial and tissue transglutaminase autoantibodies are reliable predictors of the disease, showing both good sensitivity and specificity (Sulkanen et al. 1998a, Sulkanen et al. 1998b). These tests have therefore mainly replaced the previously used gliadin antibody test in the diagnostics of coeliac disease. Endomysial and tissue transglutaminase tests may predict the disease even when the small-bowel mucosal architecture is still normal and specimens betray only mild inflammation (Troncone 1995, Kaukinen et al. 2000). The antibody tests also correlate well with coeliac-type genetics (Holm 1993b). Coeliac-type autoantibody tests may be used even in population-based screening programmes to detect undiagnosed silent coeliac disease. However, the question remains whether we are doing more good than harm in diagnosing the disease in symptomless individuals and prescribing them a life-long gluten-free diet.

The treatment of coeliac disease may be a considerable burden to the individual especially if symptoms are totally lacking. On the other hand, untreated coeliac disease may involve many complications, such as osteoporosis, infertility, neurological symptoms, autoimmune liver disorders, dental enamel defects or even malignancies ((Holmes et al. 1989, Corazza et al. 1995b, Hadjivassilliou et al. 1998, Volta et al. 1998b, Ventura et al. 1999, Martinelli et al. 2000, Vasquez et al. 2000) which might be avoided with an early diagnosis and correct treatment. It is thus important to know the true prevalence of the disorder. It is also important to understand the factors affecting the clinical picture of the disease, among them the genetic background. Further, the benefits of gluten-free diet treatment for symptomless coeliac disease patients should be thoroughly evaluated before it is possible to estimate the real benefits to be attained by screening for the disease.

This study deals with the symptomless or so-called clinically silent coeliac disease, in an attempt to arrive at answers to the above questions. We evaluated the prevalence of coeliac disease among an unselected population of schoolchildren and among a risk group of first-degree relatives of coeliac disease patients. We also studied the genetic risk factors underlying the disease. Further, we evaluated the affect of a gluten-free diet on the bone mineral density and on the quality of life of silent coeliac disease patients in order to estimate whether we are doing more good than harm by placing symptomless individuals on a life-long diet.

REVIEW OF THE LITERATURE

DEFINITION OF COELIAC DISEASE

The definition of coeliac disease has over the years referred to a permanent intolerance for dietary cereals in genetically disposed individuals, causing a typical small-bowel mucosal lesion with villous atrophy and crypt hyperplasia. The specific protein in cereals which causes this intolerance is called gluten. The mucosal lesion recovers when gluten is withdrawn from the diet.

HISTORY

Man was not originally a gluten-eater. For hundreds of thousands of years meat was the main source of nourishment and cultivation only developed as late as some 10 000 years ago. In most parts of the world the cultivation was based on non-gluten cereals. Only in a small area in South East Asia did the rivers Euphrates and Tigris offer special environmental conditions suitable for the cultivation of wild wheat and barley (Cambel & Braidwood 1970). These early grains were very different from the modern hybrids; the ears were small with few grains and their gluten content was probably low. However, concomitant with land cultivation population growth accelerated and the need gof arable land increased. Farming peoples spread from South East Asia westwards and northwards reaching first the Mediterranean and later Northern Europe. This expansion brought the previously almost unknown grains, wheat, barley, rye and oats, into the daily diet of European peoples, a development covering several thousands of years. At the same time the grains were progressively selected solely on the basis of their suitability to form dough for bread-making, and their gluten content was thereby enhanced (Feldman & Sears 1981). The industrial production of grain-containing food stuffs commenced at the end of the 16th century and during the 19th century the cereal industry started to add extra gluten to foods.

At the time when grains were introduced into the staple diet of previously meat-eating populations a significant percentage of individuals did not adapt such a dramatic change in their diet. It is highly likely that these individuals had over a period of thousands of years developed a human leucocyte antigen (HLA) -mediated immunological capacity to survive infections (Dean et al 2002) and now they reacted against gluten as they would against viruses and bacteria. For one reason or another, the reaction was targeted against the individual's own body and this led to the development of autoimmunity. During the early centuries gluten-intolerance was no great problem, since gluten-intolerant individuals were not exposed to significant quantities of gluten: the diet still consisted mainly of meat, bread ordinarily used only for ceremonial purposes and its gluten content was low. During the last few centuries, however, grains have come to constitute an increasingly important part of the diet and the gluten content of the diet has increased enormously. Gluten-intolerance has thus become an important problem of our day.

Over a hundred years ago Samuel Gee (Gee 1888) published the first detailed account in modern times of what he called the "Coeliac affection", describing "a kind of chronic indigestion, which is met with in persons of all ages, yet is especially apt to affect children between 1 and 5 years old". He described the classical form of the disease, with loose stools, weight loss and failure to thrive, and it is more than likely that many infants at that time died without proper treatment. About 60 decades later paediatrician Dicke (Dicke 1950) found the harmful effect of gluten and the treatment for gluten-intolerance was discovered. However, the means of confirming the diagnosis remained a problem; there were at that time a wide range of other diseases giving the same kind of clinical picture, but not responding to a gluten-free diet. Only in late the 1950s, after the construction of the peroral intestinal biopsy apparatus, was the connection between the finding of flat small intestinal mucosa and coeliac disease discovered.

DIAGNOSTIC CRITERIA

The diagnostic criteria for coeliac disease are based on the findings in a small-bowel biopsy specimen. The first official diagnostic criteria for coeliac disease were issued by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) in 1970. The criteria included three small-bowel biopsies: the finding of a structurally abnormal small-bowel mucosa in a patient ingesting gluten, recovery of the mucosal lesion during a gluten-free diet and biopsy-proven deterioration of the mucosa upon gluten challenge (Weijers et al. 1970). These stipulations were reconsidered in 1990 (Walker-Smith et al. 1990). The requirement of gluten challenge and second small-intestinal biopsy were relaxed. According to the present criteria the diagnosis of coeliac disease can be based on a single small- bowel biopsy specimen if the patient has typical symptoms of the disease, the small-bowel mucosal lesion is indisputable and the symptoms disappear on a gluten-free diet. However, in cases of uncertain initial diagnosis, i.e. in asymptomatic patients, in children younger than two years living in communities where other causes of enteropathy occur, or in patients in whom the findings in the first biopsy specimen are equivocal, a second biopsy and gluten challenge are recommended.

SEROLOGIC TESTS

Mucosal surfaces are the major sites in the body where foreign antigens are encountered; 80% of all immunogobulin-producing cells in humans are located in the intestinal mucosa and most of these are engaged in the production of dimers of IgA. When gluten induces an immunological reaction in the gut surface, different kinds of circulating antibodies become detectable in peripheral blood samples and can thus be used as markers of incipient immunological reaction.

The diagnosis of coeliac disease is strongly supported if during diagnosis the the blood sample is found to contain circulating coeliac disease-specific antibodies which disappear parallel to a clinical response to a gluten-free diet. A wide range of serological methods are available for the determination of these antibodies, i.e. gliadin (AGA), endomysial (EMA) and tissue transglutaminase (tTG) antibodies. Variability in methods and test protocols has been something of an obstacle in the search for a reliable serological test protocol suitable for coeliac disease diagnostics and screening. However, a great-deal of investigation has been done in this field and nowadays standardized and validated reliable non-invasive auto-antibody tests are available for the diagnostics and screening of the disease (Stern 2000).

Gliadin antibodies

Gliadin is the protein fraction of gluten soluable in aqueous alcohol. This part of the gluten has been found to be the most toxic factor in wheat protein, being responsible for the immunologic reaction in the gut. Gliadin antibodies are frequently found in patients with untreated coeliac disease (Stern et al. 1979, Burgin-Wolff et al. 1983, Savilahti et al. 1983, Unsworth et al. 1983, Ascher et al. 1990, Grodzinsky et al. 1990, Volta et al. 1990, Mäki et al. 1991, Lerner et al. 1994, Sategna-Guidetti et al. 1995, Vogelsang et al. 1995, Bottaro et al. 1997, Carlsson et al. 1999, Hill et al. 2000). Various methods have been developed to determine these antibodies: immunoflurescence (Stern et al. 1979), enzyme-linked immunosorbent assay (ELISA) (O'Farrelly et al. 1983), diffusion-in-gel ELISA (Kilander et al. 1983), solid-phase radio-immunoassay (Ciclitira et al. 1983), and a strip ELISA test (Not et al. 1993). Presumably both IgA- and IgG-class antibodies should be tested (Troncone & Ferguson 1991). According to different studies the sensitivity of IgA-class gliadin antibodies varies from 52 to 97% and that of IgG-class antibodies from 50 to 96%. The specificity of IgA-class gliadin antibodies varies from 68 to 100% and that of IgG-class gliadin antibodies from 36 to 92% (Table 1). The overall opinion of many authors nowadays seems to be that the value of gliadin antibodies in detecting coeliac disease is low: in young children test seems to correlate satisfactorily with mucosal atrophy, whereas in adults the specificity of the test is unsatisfactory. Gliadin antibodies disappear during treatment of coeliac disease with a gluten-free diet and the antibody level rises again when gluten is reintroduced into the diet. Thus seroconversion of gliadin antibodies has been used as on indicator of mucosal relapse during the follow-up of coeliac disease patients on a gluten-free diet.

Table 1. Sensitivity and specificity of serum IgA- and IgG-class gliadin antibodies (AGA) in untreated coeliac disease.

Authors	Number		IgA-class AGA		IgG-class AGA	
	Patients	Controls	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Studies in children						
(Ascher et al. 1990)	36	92	97	92		
(Lerner et al. 1994)	34	41	52	94	88	92
(Ascher et al. 1996)	55	65	91	99	96	69
(Bottaro et al. 1997)	50	25	92	68	50	36
(Vitoria et al. 1999)	27	34	96	97		
(Wolters et al. 2002)	52	49	83	86	83	80
Studies in adults						
(Corrao et al. 1994)	44	47	91	89		
(Sategna-Guidetti et al. 1995)	100	109	55	100	78	82
(Vogelsang et al. 1995)	49	53	82	83	73	74
(Sulkanen et al. 1998b)	136	207	85	82	69	73
(Lock et al. 1999)	27	115	93	95		
(Bardella et al. 2001)	40	70	95	97		

Endomysial antibodies

The discovery of endomysial antibodies was based on knowledge of the reticulin antibodies originally detected by Seah and co-workers (1971). The serum of untreated coeliac or dermatitis herpetiformis patients contained IgA-class antibodies which were directed against silver-stain-positive reticulin fibres in connective tissue around hepatic sinusoids and blood vessels. An indirect immunofluorescence method was developed for detecting these antibodies in the connective tissue of unfixed cryostat sections of rat kidney and liver. The reticulin antibody proved sensitive and specific for untreated coeliac disease (Mäki et al. 1984) and was thus used as a diagnostic test for the disease for many years.

In 1983, Chorzelski and associates described a new IgA-class serum antibody targeted against silver-stain-positive intermyofibrillar substance of the monkey oesophagus. This substance, called endomysium, resembled reticulin, and the IgA-class endomysial antibodies were found to offer a highly sensitive and specific means of identifying untreated coeliac disease and dermatitis herpetiformis patients. The correlation between reticulin and endomysial antibodies was good (Hällström 1989). However, the testing method using monkey oesophagus as substrate was unethical and expensive and thus not suitable for mass screening. To save money and monkeys, Ladinser and colleaques (Ladinser et al. 1994) introduced human umbilical cord as a new inexpensive and sensitive substitute for monkey oesophagus in testing endomysial antibodies. The substrate used does not appear to affect the method or the results (Sulkanen et al. 1998a). The sensitivity and specificity figures for this test are good (Table 2).

Tissue transglutaminase antibodies

In 1997 Dieterich and associates (Dieterich et al. 1997) identified tissue transglutaminase as the sole endomysial autoantigen. Tissue transglutaminase is a calcium-dependent, ubiquitous intracellular enzyme which in coeliac disease is able to deamidate glutamine on gliadin immunodominant peptide to glutamic acid so that after deamidation the protein fits into the peptide binding groove of the coeliac disease associated HLA-DQ. (Molberg et al. 2000) Tissue transglutaminase belongs to a family of multifunctional cross-linking enzymes which also stabilize tissues (Greenberg et al. 1991). The enzyme is released from intracellular stores upon mechanical stress, inflammation or infection or during apoptosis. Activated endothelial cells and fibroblasts which are often exposed to mechanical stress constitute the major sources of tTG.

Dieterich and colleagues, in the aforementioned study, also demonstrated that the pretreatment of coeliac disease patient serum with tissue transglutaminase abolishes endomysial antibody staining, which would imply that tissue transglutaminase is the unknown endomysial autoantigen. Using guinea pig tissue transglutaminase as substrate in an ELISA test they also showed that all 12 coeliac disease patients but none of the seven controls evinced increased IgA immunoreactivity. Subsequently a group under Korponay-Szabo (Korbonay-Szabo et al. 2000) showed by the double-staining method that tissue transglutaminase antibody binding patterns were identical with the extracellular R1-type reticulin and endomysial binding patterns seen in coeliac patients' sera. Since these findings ELISA test based on tissue transglutaminase has been used besides the endomysial antibodies as a means of detecting untreated coeliac disease. Nowadays human recombinant tissue transglutaminase has displaced guinea pig transglutaminase, as tests based on human recombinant tissue transglutaminase have proved more reliable than those based on guinea pig tissue transglutaminase. A variety of commercial kits are available for tissue transglutaminase IgA-class antibody testing. In cases with IgA-deficiency IgG-class antibodies should be used instead. The sensitivity and specificity of these tests are good (Table 3).

Table 2. Sensitivity and specificity of endomysial antibodies in untreated coeliac disease

Authors	Number		Endomysial antibodies	
	Patients	Controls	Sensitivity (%)	Specificity (%)
Studies in children				
(Volta et al. 1991)	29	20	90	100
(Sacchetti et al. 1996)	32	42	97	100
(Bottaro et al. 1997)	50	24	96	96
(Wolters et al. 2002)	52	49	92	90
Studies in adults				
(Mäki et al. 1991)	13	109	92	95
(Ferreira et al. 1992)	21	160	100	99
(Valdimarsson et al. 1996a)	19	125	74	100
(Sategna-Guidetti et al. 1997)	104	94	95	100
(Sulkanen et al. 1998b)	136	207	93	99
(Lock et al. 1999)	24	115	100	100
(Sblattero et al. 2000)	65	160	92	
(Carroccio et al. 2002)	24	183	100	100

Table 3. Sensitivity and specificity of serum tissue transglutaminase (tTG) antibody test in untreated coeliac disease using alternatively guinea pig or human recombinant tTG as substrate in the enzyme-linked immunosorbent assay (ELISA) test.

Authors	Number		Guinea pig tTG		Human recombinant tTG	
	Patients	Controls	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
(Dieterich et al. 1998)	106	114	98	95		
(Sulkanen et al. 1998b)	136	207	95	94		
(Troncone et al. 1999)	48	63	92	98		
(Lock et al. 1999)	24	115	85	97		
(Sulkanen et al. 1998b)	79	60	92	98		
(Biagi et al. 2001a)	56	52	98	85		
(Sblattero et al. 2000)	65	160	89		99	
(Leon et al. 2001)	107	72	98	99	98	100
(Bonamico et al. 2001b)	62	56	90	100	100	100
(Wolters et al. 2002)	52	49	96	92	96	100
(Castellino et al. 2000)	123	84			100	100
(Hansson et al. 2000)	22	45			100	98
(Fabiani & Catassi 2001)	399	432			90	96
(Carroccio et al. 2002)	24	183			100	97

SMALL-BOWEL MUCOSAL MORPHOLOGY

In coeliac disease gluten induces small-bowel mucosal damage which is mainly in the proximal part of the gut but may involve the whole small-bowel mucosa. The introduction of the peroral jejunal biopsy technique in the late 1950s provided a test for distinguishing gluten-induced intestinal malabsorption from non-gluten-associated forms of malabsorption. The biopsy specimens are obtained from the proximal jejunum or from the distal duodenum. Formalin-fixed, they are stained with haematoxylin-eosin and studied under light microscopy. Proper interpretation of the intestinal histology and careful orientation of the mucosa before histological sectioning is important (Cooke & Holmes 1984).

In normal duodeno-jejunal mucosa the villi are long and slender and the columnar epithelium covering the villi is tall. The brush-border and the basement membrane are intact. In morphometric studies the villous height is over 250 μm and the ratio between villous height and crypt depth is > 3. The count of intraepithelial lymphocytes (IEL) is normal (under 20 cells per 100 epithelial cells) (Holm 1993b) Figure 1a.

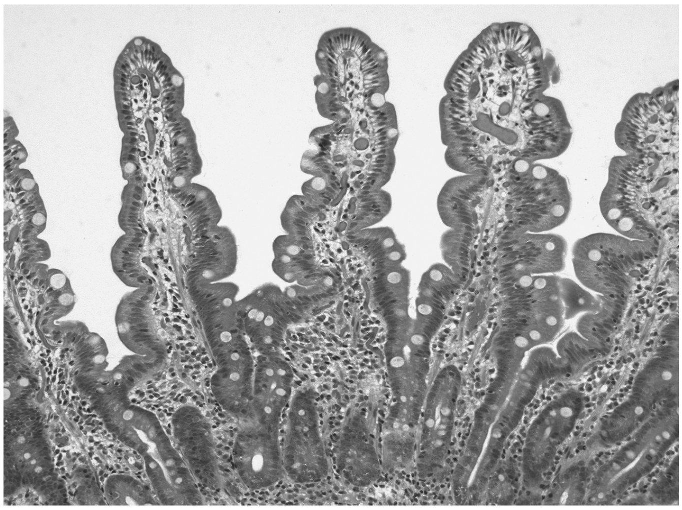
In classical flat mucosal lesion the damage involves all parts of the mucous membrane, i.e. surface epithelium, crypt epithelium and lamina propria. The major features of the flat lesion comprise markedly hypertrophied crypts and shortened villi covered by flattened enterocytes which lie on the mucosal surface between the widened crypt wells. In morphometric studies the height of the villi is not measurable and increased IEL counts (over 40 cells per 100 epithelial cells) are observed (Holm 1993b) Figure 1b.

The small-bowel mucosal lesion associated with gluten sensitivity may be a continuum from normal small-bowel mucosa to flat lesion. According to the ESPGHAN criteria the mucosal changes can be divided in five classes on the basis of morphological findings (Figure 2). According to the current criteria for coeliac disease morphological changes of class 2 – 3 are called coeliac disease. Class 4 represents total damage of the small-bowel mucosa, which may constitute a premalign or a malign lesion and is presumably associated with ulcerative jejunoileitis or lymphoma.

Figure 1.

(a) Normal mucosal architecture. High villi and normal ratio between villi height and crypt depth. No lymphocytic infiltration in epithelium or lamina propria.
(b) Small-bowel mucosal lesion in coeliac disease. Short villi and deep crypts. Lymphocytic infiltration in epithelium and lamina propria.

a



b

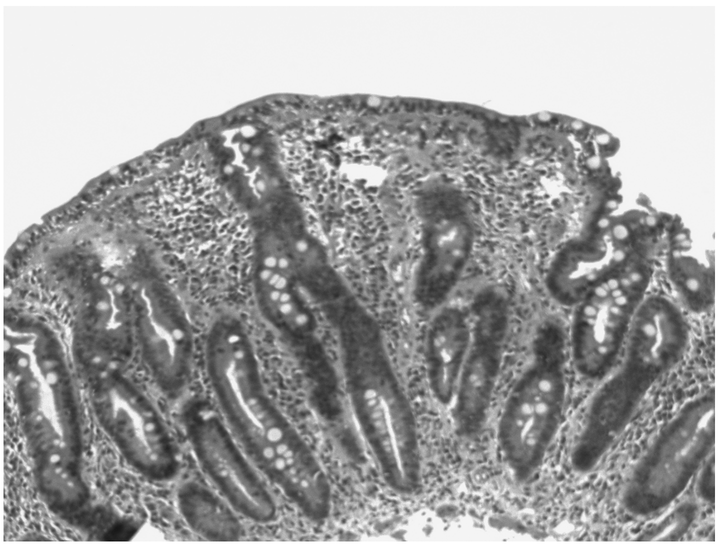
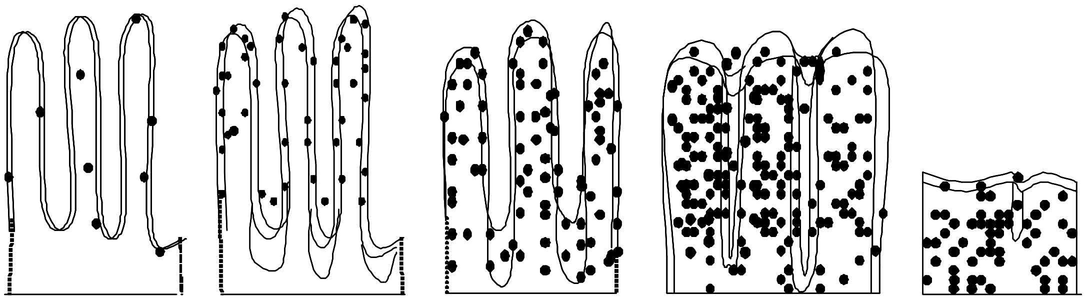


Figure 2. Five classes of small-bowel mucosal findings in normal gut and mucosal damage caused by gluten sensitivity.



class 0 class 1 class 2 class 3 class 4

Class 0 = normal mucosa
Class 1 = slight villous changes (villous height 250-300 μm)
Class 2 = partial villous atrophy (villous height 150-250 μm)
Class 3 = subtotal or total villous atrophy (villous height less than 150 μm)
i.e. flat lesion
Class 4 = atrophy of the mucosa

Mucosal T-cells

During recent years it has become apparent that the immunological process associated with the small-bowel mucosal damage in coeliac disease can also be seen in class 1 changes in mucosal specimens (Marsh 1992, Marsh 1993).

In the normal situation the intestinal immune system protects the host against intestinal pathogens (Brandtzaeg et al. 1989). Most of the IELs are T-cells and the vast majority of them are CD3 positive (Cerf-Bensussan et al. 1983). Some 70-90% of the IELs express CD8 positivity, suggesting the cytotoxic-suppressor cell function of these cells, while the remainder are mainly CD4-positive helper cells (Trejdosiewicz & Howdle 1995). Ninety per cent of IELs normally express T-cell receptor (TcR)αβ and the rest are positive for TcRγδ (Halstensen et al. 1989).

The most conspicuous phenomenon representing an on-going immune response to gluten is an increased density of IELs. However, such an increase is not pathognomic only for coeliac disease but can be found in a variety of intestinal diseases (Spencer et al. 1991), including cow´s milk intolerance (Kuitunen et al. 1982) and Crohn´s disease (Söderström et al. 1996). The increase in IELs is nontheless most striking in patients with coeliac disease (Kuitunen et al. 1982). The increased intraepithelial lympocyte population in coeliac disease consists mainly of CD3/CD8/TcRαβ-positive lymphocytes (Feguson et al. 1976, Corazza et al. 1984, Brandtzaeg et al. 1989), but also the number of TcRγδ-positive cells is increased (Halstensen et al. 1989, Spencer et al. 1989, Trejdosiewicz et al. 1991). The TcRγδ-positive cells preceed the developing mucosal damage and are often used as markers of coeliac disease latency (Iltanen et al. 1999a). However, an increased density of TcRγδ-positive cells does not fullycorrelate with the genetic haplotypes connected to coeliac disease, so that these cells are not specific to coeliac disease.

CLINICAL PICTURE

The current criteria for the condition may give the false impression that coeliac disease is a purely gastrointestinal disorder with manifest small-bowel mucosal lesions. The reality known today is much more complex. If only small-bowel mucosal lesions are considered, the magnitude of the damage can vary significantly, comprising a continuum from very mild alterations to severe villous atrophy and crypt hyperplasia. Gastrointestinal symptoms may likewise vary from very mild to severe malabsorption with overt diarrhoea. Thereafter several extraintestinal manifestations may obtain with or without gastrointestinal symptoms and may already be present in very early stages of the mucosal damage.

Classical presentation of coeliac disease

Classically coeliac disease was a disease of small children under two years of age. The most striking symptoms were gastrointestinal: diarrhoea, malnutrition and failure to thrive. The patients often suffered from iron deficiency anaemia and needed long hospitalization. During the last twenty years, however, the clinical picture of the disease has completely changed. Newly diagnosed patients are mainly adults, the symptoms are apparently milder and extraintestinal symptoms are usual.

Silent coeliac disease

In the silent form of the disease the subject has a manifest small-bowel mucosal lesion without any of the gastrointestinal or other symptoms related to coeliac disease. Some authors have expressed doubts as to the existence of this form of the disease (Johnston et al. 1998, Tursi et al. 2001). In their opinion silent coeliac disease is nothing but an undiagnosed condition and they hold that almost all coeliac disease patients have at least ekstraintestinal symptoms of the disease. However, many screening studies have exposed a selected population of patients who experience no symptoms despite manifest coeliac-type mucosal lesion (Gomez et al. 2001, Hovell et al. 2001, Volta et al. 2001a). The prevalence of this silent form of the disease is not known. Further, it is not clear whether these individuals are at risk of extraintestinal complications of the disease such as osteoporosis, infertility, neurological disorders or liver diseases, but some studies suggest that this is the case (Lindh et al. 1992, Mazure et al. 1994, Collin et al. 1996, MacGowan et al. 1996, Kolho et al. 1999, Meloni et al. 1999b, Volta et al. 2001a). It is also possible that coeliac disease may predispose individuals to other autoimmune diseases, and it has been surmised that early detection of the disease and a strict gluten-free diet might prevent other autoimmune diseases (Ventura et al. 1999).

Latent coeliac disease

Latent coeliac disease is in other words incipient coeliac disease. In this disease entity the small-bowel mucosal architecture is still intact but there is already ongoing overt inflammation in the gut wall (Weinstein 1974). The coeliac disease-specific autoantibodies may be present in the peripheral blood and an increased number of intraepithelial lymphocytes are observed in the small-bowel mucosal specimen. Especially $\gamma\delta$-specific T-cells are overrepresented, which is a phenomenon typical for coeliac disease (Corazza et al. 1983, Savilahti et al. 1990, Iltanen et al. 1999b). This latent form does not fulfil the current criteria for the disease, including small-bowel villous atrophy and crypt hyperplasia, but several studies have shown that when individuals with the latent form of the disease are followed up many are seen to develop manifest disease within a few years (Mäki et al. 1990, Collin et al. 1993, Troncone 1995). It is not known exactly how long it takes for the manifest disease to develop once inflammation has set in and what factors besides gluten really influence the onset of the disease.

Extraintestinal manifestations and complications

Dermatitis herpetiformis

The best known extraintestinal manifestation of coeliac disease is dermatitis herpetiformis. This skin disorder is characterized by a pruritic, blistering rash and the diagnosis is based on pathognomonic immunoglobulin IgA deposits in the papillary dermis in skin biopsies. Less than 10% of dermatitis herpetiformis patients present gastrointestinal symptoms, although 70% of them have gluten-sensitve enteropathy (Collin et al. 1997). The rash responds to gluten withdrawal (Reunala et al. 1984).

Iron deficiency anaemia

Isolated iron deficiency anaemia is a common complication of coeliac disease (Howard et al. 2002, Ransford et al. 2002) and may be the very first sign of the disease long before the other manifestations appear. Iron deficiency anaemia is totally reversible by gluten-free diet treatment.

Short stature

In children, short stature (Cacciari et al. 1983, Stenhammar et al. 1986) is also a well known complication of coeliac disease, being again possibly the only manifestations. Growth becomes normal on the appropriate diet.

Osteopoenia and osteoporosis

The bone mineral density of coeliac disease patients, both adults (Mazure et al. 1994, Walters 1994, Corazza et al. 1995b, Valdimarsson et al. 1996b, Kemppainen et al. 1999, Kalayci et al. 2001, Meyer et al. 2001) and children (Scotta et al. 1997, Mora et al. 1998), is decreased in 30-40% of the cases even when no signs of malabsorption are detectable. Coeliac disease is also overrepresented in patients with overt osteoporosis (Lindh et al. 1992, Mazure et al. 1994, Nuti et al. 2001). The mechanism underlying reduced bone mineral density is not fully understood. During normal bone remodelling the rate of supply of new osteoblasts and osteoclasts and the timing of the death of osteoclasts, osteoblasts and osteocytes by apoptosis are critical determinants in bone turnover. In coeliac disease the fine balance among these components may be disrupted, leading to impaired bone mineral density (Weinstein & Manolagas 2000). Disturbed calcium metabolism and primary or secondary hyperparathyreoidism may also play a role in the development of osteopoenia

(Corazza et al. 1995b). Impaired bone mineral density and osteopoenia may involve an increased risk of fractures in untreated coeliac disease patients (Vasquez et al. 2000, Fickling et al. 2001). The density seems to recover under gluten-free diet treatment (Cammarota et al. 1996, Valdimarsson et al. 1996a, Mora et al. 1998, Kemppainen et al. 1999, Castellino et al. 2000, Kalayci et al. 2001).

Bone mineral density is dependent on age and sex. The density is higher in men than in women. It is highest in individuals around 30 years of age but decreases about 0.5% each year. During the years after menopause bone mineral density decreases 2% each year. The bone mineral density of the forearm can be measured using single photon absorptiometry (SPA) (Christiansen et al. 1975) and in the spine and hip using dual energy X-ray absorptiometry (DEXA) (Cullum et al. 1989). These methods offer fairly good accuracy (5%) and high precision (1-2%). Bone mineral density may be defined as t-scores or as z-scores.

T-score is the deviation of the individual value from the normal mean value of young adults of the same sex expressed in number of standard deviations. World Health Organization has defined osteopoenia as a t-score between -1 and -2.5 and osteoporosis as a t-score less than -2.5 (WHO 1994).

Z-score is defined as the deviation of an individual measured value from the age- and sex-matched normal mean value expressed in number of standard deviations.

Bone mineral density is inversely correlated to the risk of fractures. In a prospective study a z-score of -2 was found to increase the risk of fragility fracture of the hip 4-5 fold (Cummings et al. 1993).

Neurological disorders

Neurological disorders in coeliac disease patients include gluten ataxia (Hadjivassilliou et al. 1998, Luostarinen et al. 2001), cerebral calcifications with occipital lobe epilepsy (Gobbi et al. 1990, Bernasconi et al. 1998, Luostarinen et al. 2001) and dementia (Collin et al. 1991). Seven per cent of all untreated celiac disease patients are diagnosed on the basis of various neurological symptoms (Luostarinen et al. 1999). The pathogenic mechanisms underlying neurological disorders remain obscure, but immunological mechanisms are implicated. Unlike osteoporosis, neurological symptoms seem not to recover in response to a gluten-free diet.

Infertility

Infertility is also one of the complications of coeliac disease. Usually women with untreated coeliac disease have a shorter reproductive period, are relatively infertile, and have a higher incidence of spontaneous abortions than those on a gluten-free diet (Ferguson et al. 1982, Molteni et al. 1990, Sher & Mayberry

1996). Infertility may also be the first sign of coeliac disease (Collin et al. 1996, Meloni et al. 1999b), and gluten-free diet treatment mayprove efficacions. Untreated disease may also cause insensitivity to androgens in men and lead to hypogonadism, infertility and sexual dysfunction, these again being reversible for gluten-free diet treatment (Green et al. 1977, Farthing et al. 1982, Farthing & Dawson 1983).

Liver disorders

Even 30% of coeliac disease patients evince a transient elevation of liver enzymes at the time of diagnosis (Volta et al. 1998a, Farre et al. 2002). Some develop autoimmune hepatitis (Volta et al. 1998b, Arvola et al. 2002) or primary biliary chirrosis (Floreani et al. 2002), autoimmune cholangitis or primary sclerosing cholangitis (Volta et al. 2002), and coeliac disease is also overrepresented in the population with end-stage liver failure and liver transplantation (Kaukinen et al. 2002a). The mechanisms behind the liver disorders associated with coeliac disease are still unknown, but it has been suggested that the immunological process ongoing in the gut may equally affect the liver. There is evidence that hepatic failure may be reversed on a gluten-free diet (Kaukinen et al. 2002a).

Alopecia areata

Alopecia areata has been shown to be connected to coeliac disease (Corazza et al. 1995a) but controversy persists as to the effect of gluten-free diet treatment on hair-growth (Corazza et al. 1995a, Bardella et al. 2000).

Dental enamel defects

Dental enamel defects the permanent teeth develop if a child has untreated coeliac disease during the calcification of the permanent teeth. The calcification process normalizes in response to gluten-free diet treatment.

Malignancies

The most severe if rare complications of untreated coeliac disease are malignancies. The most common of these is small-bowel T-cell lymphoma (Cooper et al. 1982, Askling et al. 2002, Catassi et al. 2002), which is diagnosed in 0.5% of coeliac disease patients. It is never found in children or adolescents and almost always it is connected to poor compliance or delayed diagnosis of the disease. Only very few coeliac disease patients suffer from the disorder called refractory sprue, which is a gluten-sensitive disorder reacting poorly to strict gluten-free diet treatment (Wahab et al. 2002). These individuals run a higher risk of small-bowel lymphoma and other malignancies (Cellier et al. 2000).

Associated diseases

Coeliac disease is associated with many other autoimmune disorders (Collin et al. 2002). According to an analysis of 26 studies the average prevalence of coeliac disease in insulin-dependent diabetes mellitus is 4.5% (Holmes 2002). In autoimmune thyroiditis the prevalence figures vary from 3.2% (Valentino et al. 1999, Volta et al. 2001b) to 7.8% (Larizza et al. 2001), and in rheumatoid arthritis the prevalence figure is 0.6% (Francis et al. 2002). Recent reports show that coeliac disease may also be associated with autoimmune myocarditis (Frustaci et al. 2002) dilated cardiomyopathy and end- stage heart failure (Prati et al. 2002).

Individuals with Down's syndrome, Turner's syndrome and Williams' syndrome also carry an increased risk of coeliac disease; 4.6% (Bonamico et al. 2001a) to 16 % (Carlsson et al. 1998) of patients with Down's syndrome, 5.0% of Turner's syndrome patients (Ivarsson et al. 1999b) and 9.5% of Williams' syndrome patients (Giannotti et al. 2001) suffer from coeliac disease.

There is debate as to the co-existence of coeliac disease and allergic diseases. A recent study suggests that the cumulative incidence of asthma in children with coeliac disease (24.6%) is significantly higher than in children free of it (3.4%) (Kero et al. 2001).

Coeliac disease is also associated with selective immunoglobulin A (IgA) deficiency (Savilahti et al. 1971, Savilahti et al. 1985, Collin et al. 1992, Cataldo et al. 1997), which is a rather rare condition, the overall prevalence in Finland being 1:400 (Koistinen 1975). About 5% of endomysial antibody-positive individuals (Prince et al. 2000) and 2.6% of biopsy-proven coeliac disease patients (Cataldo et al. 1998) suffer from selective IgA deficiency. This deficiency is an important disorder to keep in mind in the context of coeliac disease, since in IgA deficiency patients IgA-class antibody tests remain negative and IgG-class antibodies should be tested.

EPIDEMIOLOGY

In Europe the prevalence of coeliac disease was previously estimated to be 1:1000. Nowadays population-based screening studies show the overall figure for Europe to be around 1:200 – 1:300 (Table 4). This increase is to be explained mainly by the changed clinical picture of the disease: the severe malabsorption and failure to thrive are now rare findings and the multiplicity of the disease is obvious. If only patients with gastrointestinal symptoms are screened the prevalence figures are lower than those obtained from population-based screening studies. If screening is extended to risk groups such as first-degree relatives of coeliac patients and if individuals with extraintestinal manifestations are also taken into account, the prevalence figures indeed approach those of population-based screening studies.

In the United States estimaties of the prevalence vary; previously the disease was considered to be very rare but more recently prevalence figures consistent with those in Europe have been published (Not et al. 1998, Fasano et al. 2003). Likewise in South America, Australia and New Zealand, where the greater part of the population is of European origin, the prevalence figures approximate those from Europe (Table 4). The highest prevalence figures are obtained among Saharawi children living in refugee camps in Northern Africa (Catassi et al. 1999), whereas among yellow races in Asian countries and among black people in Africa and the United States the disease is almost absent.

Table 4. Prevalence figures for coeliac disease. CD = coeliac disease, AGA = gliadin antibodies, EMA = endomysial antibodies, tTG = tissue transglutaminase antibodies. DM = type I diabetes mellitus.

Authors	Country	N	Antibodies used	Prevalence of antibody positivity	Prevalence of biopsy proven CD
Screening of individuals with gastrointestinal symptoms					
(Collin et al. 1997) [1)]	Finland		AGA, EMA		1:370
(Fitzpatrick et al. 2001) [2)]	Canada	92	EMA	1:92	
Population-based screening in children					
(Korponay-Szabo et al. 1999)	Hungary	427	EMA	1:71	1:85
(Catassi et al. 1999)	Saharawi	989	EMA	1:18	
(Csizmadia et al. 1999)	Netherlands	6127	EMA	1:82	1:198
(Meloni et al. 1999a)	Italy	1607	AGA,EMA	1:70	1:95
Population-based screening in adults					
(Not et al. 1998)	U.S.A	2000	EMA	1:250	
(Kolho et al. 1998)	Finland	1070	EMA	1:97	1:130
(Ivarsson et al. 1999a)	Sweden	1894	EMA	1:172	1:189
(Rostami et al. 1999)	Belgium	1000	EMA	1:330	1:500
(Cook et al. 2000)	New Zealand	1064	EMA	1:87	1:82
(Volta et al. 2001a)	Italy	3483	EMA	1:174	1:205
(Gomez et al. 2001)	Argentina	2000	AGA, EMA	1:167	1:181
(Hovell et al. 2001)	Australia	3011	EMA	1:251	1:335
(Shamir et al. 2002)	Israel	1571	tTG, EMA	1:126	1:157

1) gastrointestinal symptoms of adults 2) recurrent abdominal pain of children

GENETICS

HLA

Coeliac disease is a genetically complex multifactorial immunomediated disease. The reported high concordance rate (70%) among monozygotic twin supports the conception that coeliac disease is a heritable disorder, but would also imply that environmental factors play an important role (Polanco et al. 1981, Greco et al. 2002). Accumulating evidence shows that the major genetic factors conferring susceptibility to coeliac disease are located in the HLA gene complex on the short arm of chromosome 6. The HLA molecules encoded by these genes are membrane-bound glycoproteins which are expressed as heterodimers of α and β chains. The principal biological function of HLA molecules is to bind peptide fragments of processed protein antigens and present them to T cells. The binding site of a peptide is in a cleft formed by the membrane distal part of the HLA molecule. Different HLA molecules bind different sets of peptides.

The HLA-molecule which confers the primary disease susceptibility in coeliac disease is the HLA-DQ2 heterodimer encoded by the alleles DQA1*05 and DQB1*02 (Sollid et al. 1989). Ninety per cent of coeliac disease patients have these alleles (Sollid & Thorsby 1990). The alleles encoding the α and β chains of the DQ2 heterodimer may be in *cis,* meaning that they are located on the same chromosome (DR3 haplotype), or the functionally same DQ2 molecule may be formed in *trans,* when the alleles come from different chromosomes (DR7/DR11 haplotype). The frequency of DQ2 in most European populations ranges from 7 to 15% (Lampis et al. 2000).

DQ2-negative coeliac disease patients have either the DR4-DQ8 haplotype, where the DQ8 molecule is coded by DQA1*03 and DQB1*0302, or either the DQA1*05 or DQB1*02 part of the DQ2 heterodimer (Spurkland et al. 1992). Other HLA-haplotypes connected to coeliac disease are exceptional especially in Finnish population: only 0.7% of coeliac disease patients do not carry any of these haplotypes. (Polvi et al 1998).

Numerous studies indicate that DQ2 homozygous individuals carry six times higher risk of coeliac disease than the heterozygous (Ploski et al. 1993, Howell et al. 1995, Polvi et al. 1996). It is claimed that the number of risk alleles carried by the patient correlates to the time of onset and the severity of the disease (Congia et al. 1994, Howell et al. 1995, Polvi et al. 1996). There is also evidence that some HLA-haplotypes might be protective against the disease (Ploski et al. 1993, Howell et al. 1995, Polvi et al. 1996), but overall the role of the different HLA haplotypes in determinating the clinical outcome of the disease is still unknown.

Table 5. Results of genomewide linkage analysis studies of coeliac disease risk factors outside the human leukocyte antigen (HLA) region.

Author	Country	Gene locus											
(Zhong et al. 1996)	Ireland				6p23	7q31.3		11p11	15q26				22cen
(Greco et al. 1998a)	Italy			5qter				11qter					
(King et al. 2000)	United Kingdom				6p12			11p11		17q12	18q23		22q13
(Liu et al. 2002)	Finland		4p15	5qter									
(Naluai et al. 2001)	Scandinavia	2q11-13		5q31-33			9p21	11q23-25 11p15		17q22			
(Popat et al. 2002)	Northern Europe		4p14									19p13.3	
(Woolley et al. 2002)	Finland								15q11-13				

Other candidate genes

Risch and colleagues (Risch 1987) and Bevan and colleagues (Bevan et al. 1999) have suggested that the HLA locus may not be the only determinant of familial aggregation in coeliac disease. This concepiton is supported by the finding that the concordance rate among HLA identical siblings is only 30% (Mearin et al. 1983). Therefore genomewide linkage analysis has been used as a means of detecting genes outside the HLA locus which might play a relevant role in determining the clinical appearance of the disease. Several candidate regions have been proposed among different populations (Table 5), but the results are variable.

GLUTEN-FREE DIET TREATMENT

Gluten can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and other soluable constituents. Gluten is the component that is responsible for the uniqueness of wheat with respect to its baking quality. Gluten potein is unique in terms of its aminoacid composition: it contains high amounts of glutamine, proline and hydrophobic aminoacids but the content of aminoacids with charged side groups is low. The major protein fractions of gluten in wheat are gliadin which is soluable in aqueous alcohol and glutenin which is non-soluable. The components of gliadin can further be classified into three groups (α-, γ- and ω-gliadins) on the basis of mobility at low pH in gel electrophoresis. Proline-rich α-gliadin has shown to be the toxic part of gluten (Cornell et al. 1992) In common the alcohol soluable protein fraction of different cereals are called prolamins. In rye the prolamin fraction is called secalin, in barley hordein and in oats avenin.

The only known treatment of coeliac disease is a life-long gluten-free diet. The forbidden cereals are wheat, rye and barley. However, despite of similarity in prolamin fraction, oats seems to differ from other cereals in context of ability to cause coeliac disease. In 1995 Janatuinen and colleagues discovered that coeliac disease patients can tolerate oats. A five-year follow-up has shown up that there is no mucosal inflammation in small bowel specimen of coeliac disease patients eating oats daily (Janatuinen et al. 2002). Also patients with dermatitis herpetiformis tolerate oats (Hardman et al. 1997, Reunala et al. 1998).

On a strict gluten-free diet the small- bowel mucosal lesion heals mostly within a year (Kaukinen et al. 1999). However, the definition of a strict gluten-free diet remains vague, i. e. the highest daily intake of gluten permissible for coeliac patients is a matter of debate. In some studies regular consumption of 5g of gluten daily has not caused mucosal damage (Kumar et al. 1988, Montgomery et al. 1988), whereas other studies show that some individuals may evince symptoms even when adhering to very strict diets (Catassi et al. 1993, Troncone

1995, Ellis et al. 1998). It is thus obvious that gluten sensitivity differs among individuals. The Codex Alimentarius standard stibulates that foods labeled gluten-free must contain less than 0.05 g nitrogen per 100g of food products on a dry matter basis (Joint FAO/WHO Food Standards Programme (1981). This means that wheat starch-based gluten-free flours meeting this standard may contain 40-60 mg of gluten/100g of cereals. Some authorities insist that the permitted amount of gluten should be even lower. However, the precise quantification of potentially toxic cereal proteins possibly present in foods is at present impossible. Groups under Kaukinen (Kaukinen et al. 1999) and Selby (Selby et al. 1999) in two different studies compared the mucosal integrity of individuals on a strict wheat starch-containing diet to that in individuals on a strict naturally gluten- free diet and found no difference in favour of a naturally gluten-free diet. These findings suggest that small amounts of gluten are not harmful to the small-bowel mucosa of coeliac disease patients, and on this basis many experts consider a daily gluten consumption of <50 mg safe.

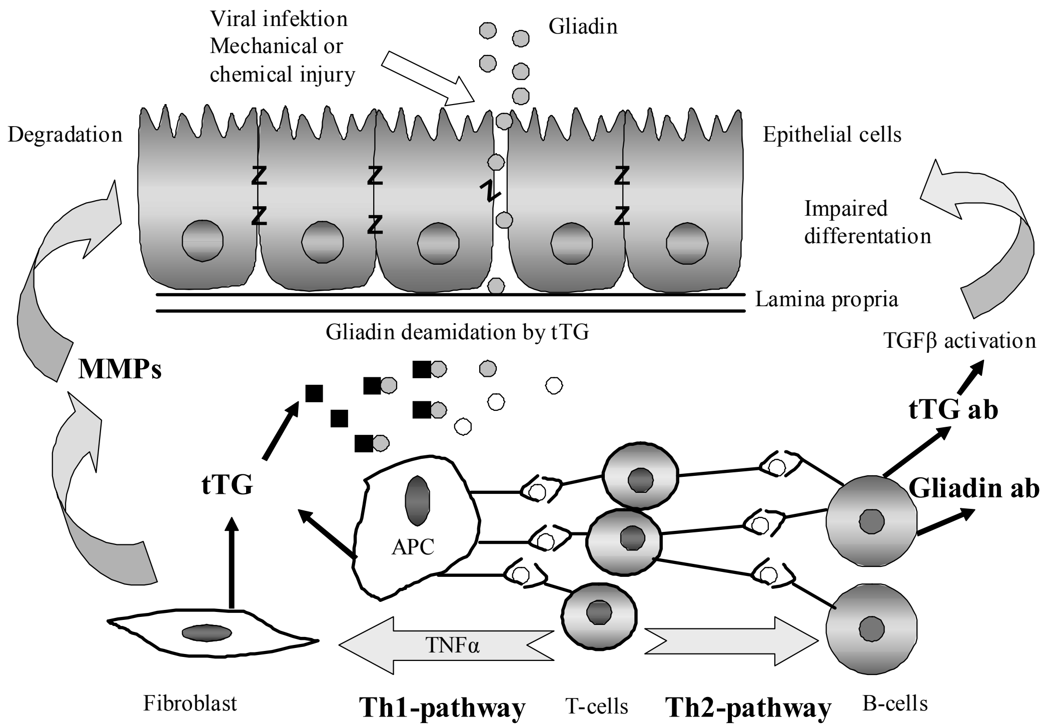
A gluten-free diet may often be difficult to maintain and the selection of gluten-free products may be poor. The compliance of coeliac disease patients is thus often questionable, as shown by many reports; only 17-65% of adult patients adhere to a strict gluten-free diet. (Kluge et al. 1982, Kumar et al. 1988, Mayer et al. 1991) A special group at risk of dietary transgressions is the group of symptomless screen-detected coeliac adolescents. Fabiani and associates (Fabiani et al. 1996) studied the compliance of such patients and found that 52% adhered to a strict gluten-free diet while 48% reported occasional transgressions. However, it is of note that despite occasional transgressions 19 out of 24 studied adolescents were negative for the endomysial antibodies widely used in the follow-up of mucosal recovery during a diet. Thus, the consequences of occasional transgressions of the gluten-free diet remain unclear. Five-year follow-up showed that the compliance of screen-detected patients was lower than that of symptom-detected patients (Fabiani et al. 2000). Even 32% of silent patients yielded positive endomysial antibody test results revealing poor compliance.

PATHOGENESIS

The pathogenetic mechanisms underlying coeliac disease are still largely unknown, though charting of them has recently made rapid progress. Nowadays the disorder is considered to be a result of a complex interplay of genetic and environmental factors. The known factors affecting the presence of the disease are gluten and genetic background. The time-point of the first gluten exposure and the amount of gluten consumed seem to play a prominent role in disease manifestation (Weile et al. 1995, Ivarsson et al. 2002). Viral infections such as adeno-virus infection may also be important for the onset of the disease (Kagnoff et al. 1984, Lähdeaho et al. 1993). However, the process inducing the onset of the disease is still asubject of intensive research.

Small amounts of dietary proteins, including gliadins, can reach the lamina propria of epithelial cells in the small- bowel mucosa, especially when mucosal integrity is impaired as a result of intestinal infection or mechanical or chemical injury. Here also zonulins, proteins involved in connecting endothelial cells, may play a role. (Fasano et al. 2000) In individuals genetically predisposed to coeliac disease, antigen- presenting cells, such as macrophages and dendritic cells carry specific antigen- binding sites on their surfaces. These antigen-binding sites are formed by class II HLA molecules which include a gliadin-binding groove. The antigenic part of gliadin does not, however, fit the groove without preliminary processing. Tissue transglutaminase, an enzyme released from macrophages and fibroblasts, is able to deamidate glutamine on gliadin immunodominant peptide to glutamic acid so that after deamidation the protein fits into the peptide binding groove of the coeliac disease associated HLA-DQ. (Molberg et al. 2000) This elicits aproliferative response of gliadin-reactive T cell clones (Arentz-Hansen et al. 2000). These, mainly T-helper (Th) cells, can cause a variety of reactions: The Th1-type reaction conducts release of tumour necrosis factor α (TNF α), which is a powerful inducer of matrix metalloproteinase (MMP) expression and activation in intestinal fibroblasts. It has been suggested that these MMPs may cause matrix breakdown and lead to villous atrophy (Schuppan 2000). The Th2-type reaction induces the activation of B-cells which produce antibodies against gliadin and against tissue transglutaminase. Coeliac disease specific tissue transglutaminase autoantibodies inhibit transforming growth factor β- mediated epithelial cell differentation and increase epithelial cell proliferation (Halttunen & Mäki 1999). Lacking differentiation of epithelial cells blocks the formation of villous structures, but the proliferation of undifferentiated cells leads to crypt hyperplasia (Figure 3).

Figure 3. Summary of theories of the pathogenesis of gliadin-induced small-bowel mucosal lesion with villous atrophy and crypt hyperplasia. ● = gliadin, ■ = tissue transglutaminase (tTG) o = gliadin deamidated by tTG, APC= antigen-presenting cell, MMP = matrix metalloproteinase, Th= T- helper cells, TGFβ= transforming growth factor beta, Z=zonulin. Gliadin is deamidated by tissue transglutaminase and introduced to antigen-presenting cells, which leads to T-cell activation. The Th1-type reaction induces matrix metalloproteinases which cause matrix breakdown. The Th2-type reaction activates B-cells to produce antibodies against gliadin and against tissue transglutaminase. These antibodies inhibit TGFβ-mediated epithelial differentiation, this leading to villous atrophy and crypt hyperplasia. Figure modified from original illustration by Schuppan and Hahn (2000).



QUALITY OF LIFE

As the limitations of traditional indicators of health have been increasingly recognized, quality of life has emerged as one important outcome of medical care. Quality of life is defined as “*an individual´s perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a broad concept affected in a complex way by the person´s physical health, psychological state, level of independence, social relationships, and their relationships to salient features of their environment”*. (WHO 1993)

In coeliac disease quality of life studies are extremely important, since life-long gluten-free diet treatment is a matter of every-day life and may strongly affect the quality of life of patients. Few cross-sectional evaluations of the quality of life of treated adult coeliac disease patients are available (Hallert et al. 1998, Hallert et al. 2002, Usai et al 2002). These studies suggest that the quality of life of these adult patients is often impaired. In children the quality of life of treated coeliac disease patients does not seem to differ from that of controls (Kolsteren et al. 2001).

Measuring quality of life

Measuring the quality of life can be approached in various ways. The literature presents a wide range of test patterns (Naughton & Wiklund 1993). Two basic types of instruments are available: generic and disease-specific questionnaires. The generic questionnaires include many dimensions of health-related quality of life and provide a universal formula for evaluating the impact of disease. They can be used to compare the effect of treatment in different medical conditions and among different studies. Generic measures are less likely to detect small but clinically important changes induced by treatment in selected patient populations. Disease-specific questionnaires address in detail areas of dysfunction induced by a particular disease, condition or treatment. Compared with generic measures, specific questionnaires are more responsive in detecting small treatment-induced changes (Wiklund 1990). Questionnaires which address a particular aspect but are still of a general nature, for example the Psychological General Well-Being index (Dupuy 1984) represent a level between generic and specific measures.

In measuring the quality of life, there is always a contradiction between the view of the patient and the researcher. The mode of administration and the environment in which a questionnaire is completed may affect the way problems are reported. It has been suggested that most symptoms tend to be reported as improved in interviews (Bulpitt et al. 1974). On the other hand some clinicians disapprove of patients ratings because of their subjectivity. Poor correlations between professionals´ and patients´ assessments have been reported (Jachuck et al. 1982). Nowadays self-administered questionnaires are preferred (Lydick & Epstein 1993).

Another important question in measuring the quality of life is that of meaningfulness in respect of changes in it. There are various interpretations regarding the clinical impact of a change in quality of life. These interpretations can be divided into two categories: distribution-based and anchor-based (Lydick & Epstein 1993).

Distribution-based interpretations are based on the statistical distributions of the results obtained from a given study. Most of these interpretations are permutations of the means and standard deviations of changes seen in a particular study or comparisons of study results to the means or standard deviations of some reference population. The most commonly cited of these measures is the magnitude of effect in which the importance of the change is scaled by comparing the magnitude of the change to the variability in stable subjects, for example on the baseline of untreated healthy individuals. The anchor-based category of interpretations of clinical impact represents instances where changes seen in quality of life measures are compared to other clinical changes or results. Regardless of the mode interpretation of results the most important aspect in terms of meaningfulness is the follow-up period. Repeated trials with intervention should include a follow-up time long enough (at least 3-6 months) to allow changes in the quality of life to take place. The consideration of the influence of confounding factors is also essential for the reliability of the results of quality of life studies (Wiklund 1990).

PRESENT STUDY

PURPOSE OF THE STUDY

To evaluate

the prevalence of silent coeliac disease in the general population (**I**) and in a well-known risk group (**II**).

the correlations between coeliac disease specific antibodies, small-bowel biopsy findings and the HLA haplotypes connected to coeliac disease (**I, II**)

the HLA connections in coeliac disease, especially in silent coeliac disease (**I, III**).

the influence of gluten-free diet treatment on the bone mineral density of silent coeliac disease patients (**IV**).

the influence of gluten-free diet treatment on the quality of life of silent coeliac disease patients (**V**).

SUBJECTS AND STUDY PROTOCOLS

*Screening studies **(I, II)***

*Schoolchildren population (**I**)*

Initially, in 1994, a sample of 4280 schoolchildren aged 7-16 years were invited to join a study of diabetic risk factors (Kulmala et al. 2001). The cohort included all schoolchildren of this age living in five municipalities in northern Finland (Haapajärvi, Ii, Oulainen, Yli-Ii and Ylikiiminki) and represents 0.7% of the whole Finnish school population of this age. Altogether 3662 individuals (85.6%) consented to blood sampling. In 2001 we took advantage of the existing material and designed a coeliac disease screening study. The volume of eight serum samples was not sufficient for analyses and thr individuals in question were excluded the remaining study cohort for the coeliac disease screening study numbering 3654 individuals (1826 males), median age 12 years at initial sampling.

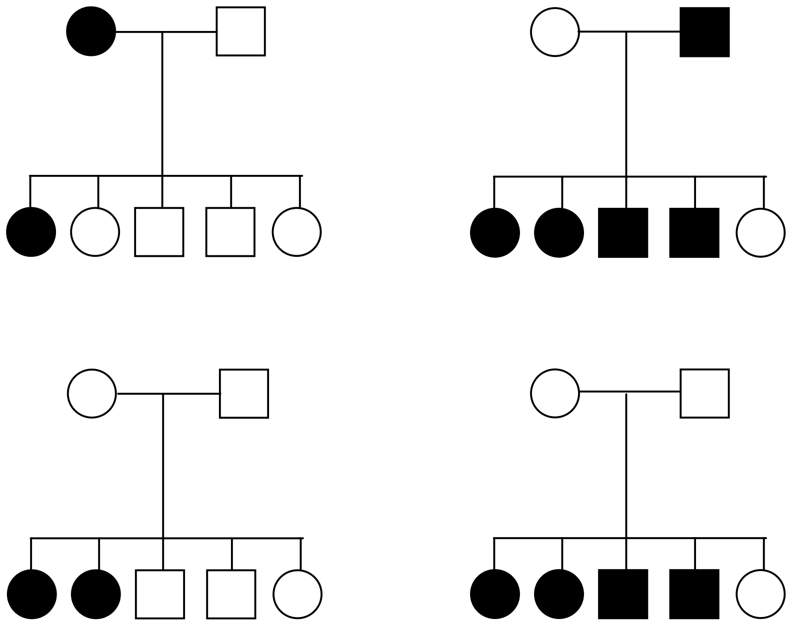
The serum samples collected in 1994 were in 2001 tested under code simultaneously for endomysial antibodies in Tampere, Finland and for tissue transglutaminase antibodies in Freiburg, Germany. HLA-testing was carried out on 3627 individuals for whom whole-blood samples were available. All antibody- positive individuals, if not already diagnosed for coeliac disease, were recommended for upper gastrointestinal endoscopy. At the same time a second blood sample was drawn and clinical symptoms asked by a semistructured questionnaire. The diet was checked by a clinical dietitian.

*Multiple-case coeliac families (**II**)*

The initial material for study **II** was collected by advertising in the Finnish Coeliac Society newsletter and calling for multiple case coeliac families to join the study. Multiple case families were chosen since we wanted to use the material also for genetical studies. Altogether 161 families were recruited of which 137 fulfilled our inclusion criteria. These families comprised at least two previously diagnosed coeliac disease or dermatitis herpetiformis patients and at least one healthy first-degree relative. Families with coeliac disease only in the parents as well as families without non-coeliac family members were excluded. Examples of the different kinds of families joining the study are seen in Figure 4.

Figure 4. Examples of families joining study **II**.

Open square = healthy man
Open circle = healthy woman
Shaded square = man with coeliac disease or dermatitis herpetiformis
Shaded circle = woman with coeliac disease or dermatitis herpetiformis.



The total body of family members in question comprised 872 individuals. Eighty-four (9.6%) of them refused to participate or could not be traced. Thus the final number of participants was 788 (451 females and 337 males). This material included 322 previously diagnosed patients (median age 40 years, range 3-78), of whom 274 were suffering from coeliac disease and 48 from dermatitis herpetiformis. Altogether there were 466 healthy family members (median age 41 years, range 2-90) consuming a normal gluten-containing diet. Hospital records of the previously diagnosed coeliac disease and dermatitis herpetiformis patients were scrutinized to ensure that the diagnoses were based on small-bowel or skin biopsies

All 466 healthy family members were first screened for gliadin and endomysial antibodies and, after one year under code, for tissue transglutaminase antibodies. Upper intestinal endoscopy was recommended for individuals positive for either gliadin and/or endomysial antibodies. HLA-DQ2 and DQ8 typing was performed in all gliadin- or endomysial antibody-positive healthy family members and in one of the previously diagnosed coeliac disease patients in each family.

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*Genetic study (**III**)*

In the genetic study (**III)** the material consisted of 28 sib-pairs suffering from coeliac disease (Table 6). The sib pairs were obtained from the initial family material collected for study **II**. One of the siblings had the silent form of the disease, the other the symptomatic form. Only one sibling pair from each family was included.

In order to evaluate the role of DR-DQ haplotypes in distinguishing between the clinically silent and symptomatic forms of coeliac disease, the HLA DR-DQ distribution was evaluated between siblings with the different forms of the disease.

*Bone mineral density study (**IV**)*

In the bone mineral density study (**IV**) the study group consisted of 19 clinically silent coeliac disease patients diagnosed through our routine screening programmes (Table 6). Fifteen of the patients were initially healthy family members of coeliac disease patients screened for coeliac disease in study **II**. Four had disorders not indicative of gluten enteropathy (two had oral ulcers, one had non-specific arthritis and one was suffering from ataxia).

Thirty newly diagnosed symptomatic coeliac disease patients served as controls. These cases were obtained from the Department of Medicine at Tampere University Hospital.

Bone mineral densities in clinically silent and symptomatic coeliac disease patients were measured at the time of diagnosis and after one year on a gluten-free diet.

Quality of life study (V)

The quality of life of 19 screen-detected coeliac disease patients and 21 symptom-detected patients was determined in study **V** and compared to that of 105 non-coeliac individuals (Table 6). The screen-detected patients were diagnosed among healthy first-degree relatives in study **II** and the symptom-detected patients were obtained from the Department of Medicine at Tampere University Hospital. The control material of non-coeliac individuals was collected by the Finnish Coeliac Society. One hundred and five treated adult coeliac disease patients were selected from member files. They were asked to recruit for the study an individual living in their neighbourhood and not suffering from coeliac disease or having a family history of the disease and eating a normal gluten-containing diet.

Gastrointestinal symptoms and quality of life were prospectively studied in patients with silent coeliac disease and symptomatic disease at the time of diagnosis and after one year on a gluten-free diet. The non-coeliac comparison group was studied only once, since the Ethical Committee of Tampere University, which approved the study protocol, did not approve follow-up of these participants.

Table 6. Demographic data on the participants in studies **III**, **IV** and **V**.

Study	Clinically silent patients			Clinically symptomatic patients			Healthy controls		
	n	F/M	Median age years (range)	n	F/M	Median age years (range)	n	F/M	Median age years (range)
III	28	17/11	38 (7-60)	28	21/7	37 (8-56)	-	-	-
IV	19	6/13	45 (23-69)	30	23/7	44 (21-68)	-	-	-
V	19	10/9	48 (21-71)	21	17/4	49 (19-67)	105	90/15	48 (23-87)

METHODS

Autoantibodies

*Gliadin antibodies **(II)***

The analysis for IgA- and IgG-class gliadin antibodies was performed in accordance with standard ELISA with a crude gliadin (Sigma G3375) as antigen (Savilahti et al. 1983). The results were obtained from a standard curve established according to dilutions of a positive reference serum and converted to concentrations of arbitrary ELISA units (EU/ml). Serum samples exhibiting antibodies in higher concentrations than healthy age-matched controls, plus two standard deviations, were considered positive.

*Endomysial autoantibodies **(I,II)***

IgA- and IgG-class endomysial antibodies were determined by an indirect immunofluorescence method described by Sulkanen and colleagues (Sulkanen et al. 1998a). Unfixed cryostat sections of human umbilical cord were used as antigen for endomysial antibodies. After initial screening with patient serum using titres of 1:5 and 1:50, positive sera were further titrated 1:100, 1:200, 1:500, 1:1000, 1:2000, 1:4000 and 1:8000. A serum dilution of 1:$\geq$ 5 was considered positive.

*Tissue transglutaminase antibodies **(I,II)***

In study **I** determinations of IgA-class tissue transglutaminase antibodies were carried out with Celikey® (Pharmacia Diagnostics, Freiburg, Germany), a human recombinant tissue transglutaminase-based commercial autoantibody kit, in accordance with the manufacturer´s instruction. The detection limit for the assay was 0.1 U/ml and for the present study the lower limit of the equivalent range between 5 and 8 U/ml was chosen as cut-off. Serum samples evincing probability of IgA-deficiency, i.e. samples with IgA-class tissue transglutaminase antibody levels below the detection limit, were further tested for determination of IgG-class tissue transglutaminase antibodies with a research ELISA (Pharmacia Diagnostics, Freiburg, Germany), as previously described (Hansson et al. 2002). Values above 5 U/ml were considered positive.

In study **II** IgA-class tissue transglutaminase antibodies were measured with the FDA-approved tissue transglutaminase ELISA kit (Quanta LiteTM tTG ELISA 708730, INOVA Diagnostics, Inc., San Diego, CA, USA), which is based on guinea pig tissue transglutaminase. The antibody concentrations were expressed in percentages of the positive reference serum, i.e. arbitrary units (AU), and values >20 AU were considered positive.

*Endoscopy (**I,II**)*

Small-bowel biopsies (**I, II**) were obtained with a paediatric or adult Watson capsule or with forceps from the distal duodenum during upper gastrointestinal endoscopy. During the procedure multiple duodenal biopsy samples were taken for routine histology. Orientated formalin-fixed haematoxylin-eosin-stained biopsy specimens were studied under light microscopy. Findings of severe partial or subtotal villous atrophy with crypt hyperplasia in a small-bowel specimen were considered to indicate coeliac disease. In study **I** one sample was prepared for immunohistochemical stainings.

*Morphometric studies (**I**)*

Villous height (VH) and crypt depth (CrD) were measured and the VH/CrD ratio was counted in haematoxylin-eosin stained small bowel biopsy specimens in study **I**. A ratio <2 was considered indicative of coeliac disease, i.e. villous atrophy with crypt hyperplasia.

*Immunohistochemical stainings (**I**)*

Frozen small-intestinal biopsy samples were stained for γδ TcR-bearing intraepithelial lymphocytes and cell densities were counted as previously described (Iltanen et al. 1999b). The threshold for positive γδ T-cell density was 3.5 cells/mm, which is the mean +2 standard deviations of the density in controls (Savilahti et al. 1997).

Biopsy specimens were also stained for HLA-class II expression and DR expression was considered enhanced when epithelial staining was strong or crypt expression alone was present (Iltanen et al. 1999b).

The monoclonal antibodies and the titres used are described in a paper by Iltanen and associates (Iltanen et al. 1997).

*HLA-DR-DQ typing (**I-III**)*

In study **I** HLA-typing was performed using a screening test developed to detect alleles associated with risk of and protection against type 1 diabetes (Nejentsev et al. 1999). Selected HLA-DQB1 alleles, including DQB1*02 and DQB1*0302, were first defined and samples positive for the DQB1*02 allele were further analysed for the presence of associated DQA1 allelels: DQA1*0201 and DQA1*05.

In studies **II** and **III** DQB1 typing was based on polymerase chain reaction with allele-specific primers identifying the major DQB allele-groups and carried out with a Dynal DQ “low resolution” SSP kit (Dynal A.S, Oslo, Norway). DR-DQ haplotypes were determined using two microsatellite markers DQCAR and DQCARII (Karell et al. 2000). In cases of doubt the samples were also typed by commercial LiPA HLA-DRB1 kit (Immunogenetics, Dartford, UK).

*Bone mineral density measurements (**IV**)*

The bone mineral density of clinically silent and symptomatic coeliac disease patients was measured by dual-energy X-ray absorptiometry (Norland XR 26, Norland Corp., Fort Atkinson, WI, USA) in the lumbar spine and femoral neck at the time of diagnosis and after one year on a gluten-free diet. The baseline bone mineral density data were expressed as t-scores with reference to sex-matched young normal data. We were thus able to compare the bone mineral density measurements of an individual patient during the follow-up. The percentage change in bone mineral density was calculated, and an individual change was considered significant if its magnitude exceeded $2\sqrt{2}$ times the short-term *in vivo* precision of the given measurement. In our laboratory, the significance levels were thus standard deviation + 2.0% for spine and standard deviation + 1.4% for femur (Sievänen et al. 1996).

Quality of life questionnaires (V)

Psychological General Well-Being (PGWB)

The general well being of patients and controls was measured by the Psychological General Well-Being questionnaire (Dupuy 1984) which consists of 22 items including both positively and negatively affecting states. It covers six dimensions: anxiety, depressed mood, positive well-being, self-control, general health and vitality. All questions are rated on a six-point Likert scale (from 1 to 6) with lower values indicating less satisfactory perceptions. The total index is the sum of ratings on each question and ranges from 22 to 132, higher scores indicating greater well-being.

Gastrointestinal Rating Scale (GSRS)

The gastrointestinal symptoms of patients and controls were measured by the Gastrointestinal Symptoms Rating Scale (Svedlund et al. 1988). The questionnaire comprises 15 items representing five major gastrointestinal symptoms: diarrhoea, indigestion, constipation, abdominal pain and reflux. The total index is the mean of these 15 items, varying from 0 to 6. Zero indicates the absence of and six the worst possible degree of the symptom.

Food records (V)

Coeliac disease patients in the quality of life study (**V**) were asked to complete four-day food records twice during the study: the first time the records were filled at the time of diagnosis to ensure that these patients were initially on a gluten-containing diet and the second time after one year on gluten-free diet to monitor compliance. Using these records a dietician estimated the mean gluten intake by means of the UNIDAP computer program (Unilever B.V. Rotterdam, Holland, 1994).

The coeliac disease patients' personal conception of long term compliance with the gluten-free diet was also evaluated by visual analog scale (Aitken 1969, Bowling 1995) from 0 to 100%.

Statistical analyses

In study **I** 95 per cent confidence intervals (CI) were calculated for the prevalence figures and odds ratios (OR). OR was calculated using the equation a x d/b x c and positive predictive values (PPV) using the equation a/a+c x 100, where a and c represent numbers of genotype risk factor-positive individuals with or without coeliac disease-specific autoantibodies and b and d numbers of genotype risk factor-negative individuals with or without autoantibody positivity.

In study **II** Spearman´s rank correlation coefficient was calculated between tissue transglutaminase and endomysial antibody titres (Graph Pad PRISM, version 2.01, 1996, Graph Pad Software Inc. San Diego, CA, USA).

In study **III** differences in distributions of HLA DR-DQ haplotypes were compared between silent and symptomatic coeliac disease patients using Fisher´s exact test. Student´s t-test was used to compare the median age at the time of diagnosis between the two groups of patients.

In study **IV** the significance of the difference in t-scores between different groups of patients and healthy individuals were established at 95% confidence intervals.

In study **V** statistical analysis was carried out using analysis of covariance for repeated measures. The objective in analysing covariance was to study the significance of possible confounding factors (age, gender, economic situation, body mass index). The computation was carried out on Statistica/Win (Edition ´98) software (StatSoft, Inc. Tulsa, OK, U.S.A).

Ethical considerations

The study protocol in study I was approved by the Ethical Committee of Oulu University Hospital and the protocols of studies **II-V** by the Ethical Committee of Tampere University Hospital. Written informed consent was obtained from all participants. The hospital records and questionnaires are stored according to the regulations of the European Community directives.

RESULTS

*Antibody test results, small bowel biopsies and HLA (**I,II**)*

*Schoolchildren population (**I**)*

Among 3654 schoolchildren altogether 56 individuals (1.5%) were positive for either IgA- or IgG-class endomysial and/ or tissue transglutaminase antibodies (Figure 5).

Fifty individuals were positive for both IgA-class endomysial (median titre 1:500, range 1:5 to 1:4000) and IgA-class tissue transglutaminase antibodies (median titre 70.3 U/ml, range 8.8 to 680). One was positive for IgA-class endomysial antibodies only (titre 1:5). Two were positive for only IgA-class tissue transglutaminase antibodies (titres 5.7 end 7 U/ml) and 3601 were negative for both tests. Thus, the correlation between these methodologically different autoantibody tests was good: in 3651 out of 3654 cases the results were concordant.

Seventeen of the 3654 subjects had undetectable serum levels of IgA-class tissue transglutaminase antibodies. Three of these were clearly positive for IgG-class tissue transglutaminase antibodies (titres 160.0, 148.2 and 26.4 U/ml, respectively) and all proved to be IgA-deficient.

Before the year 1994, when the blood samples were drawn, no coeliac disease cases had been diagnosed among the 3654 children involved.

Between the years 1994 and 2001 ten symptom-detected biopsy-proven cases had been diagnosed because of abdominal symptoms (Figure 5). All of these were positive for endomysial and tissue transglutaminase antibodies in their blood samples drawn in 1994. One had IgA deficiency but was positive for IgG-class endomysial and tissue transglutaminase antibodies. Seven carried the HLA DR3-DQ2 haplotype and three the HLA DR4-DQ8 haplotype.

In 2001 all antibody-positive individuals (n=46) without previously diagnosed coeliac disease were invited to attend for small-bowel biopsy and control antibody testing. Thirty-six (78%) consented to biopsy (Figure 5).

Twenty-seven individuals showed villous atrophy and crypt hyperplasia (mean VH/CrD ratio 0.87, range 0.11 to 1.68) in their small-bowel mucosal specimen. Also γδ T-cell densities were elevated (mean 20 cells/mm, range 3.5 to 71.6). Aberrant upregulation of HLA-DR expression was seen in 25/27 mucosal specimens. All of the individuals concerned carried HLA haplotypes connected with coeliac disease: 22 carried haplotype HLA DR3-DQ2, three were positive for haplotype HLA DR4-DQ8 and one was heterozygous for HLA DR3-DQ2 and DR4-DQ8. One of the individuals carried only the DQB1*02 allele (DR7 haplotype). The control serum sample drawn at the time of biopsy showed IgA- or IgG-class endomysial and tissue transglutaminase antibodies in 26 out of 27 cases (two IgA-deficient individuals included). One individual was negative for both antibody tests in the control serum sample. All newly diagnosed coeliac disease patients were prescribed a gluten-free diet.

Nine individuals showed normal mucosal architecture in their small-bowel mucosal specimen (Study **I**, Table 1). However, 5 of these had elevated γδ T-cell densities and 8 had aberrant upregulation of HLA-DR in their mucosal specimen. All but one were positive for coeliac-type HLA haplotypes: seven carried HLA DR3-DQ2 and one was positive for HLA DR4-DQ8. In the control serum sample in 2001 two of the nine individuals with normal small-bowel mucosa still showed elevated serum endomysial and tissue transglutaminase antibody levels. Five initially endomysial and tissue transglutaminase antibody-positive individuals had turned out to be negative for the antibodies in the control sample but still evinced ongoing mucosal inflammation and HLA haplotype typical for coeliac disease. Two individuals with initially only positive tissue transglutaminase antibodies showed negative antibodies in the control sample. One of these did not carry a haplotype associated with coeliac disease.

Ten of the 46 antibody-positive individuals refused small-bowel biopsy despite high endomysial (median titre 1:200, range 1:5 to 1:4000) and tissue transglutaminase (median titre 70.6 U/ml, range 4.8 to 169.7) antibody titres. Four of these consented to control serum sampling and all had elevated endomysial and tissue transglutaminase antibody titres.

*Multiple case coeliac families (**II**)*

Among 466 healthy first-degree relatives of multiple-case coeliac disease families 72 individuals (15.5%) were positive for either gliadin and/or endomysial antibodies (Figure 6). Twenty-four were positive only for IgA-class endomysial antibodies, 20 for both IgA-class gliadin and endomysial antibodies and 27 for IgA-class gliadin antibodies only. One individual was positive only for IgG-class gliadin antibodies. Thus 44 (9.4%) of the healthy family members of coeliac disease patients were positive for endomysial antibodies and 48 (10.3%) for gliadin antibodies.

Retrospectively all sera from 466 healthy family members were tested for tissue transglutaminase antibodies. Altogether 60 (12.9%) of them proved positive. When compared to the results of the endomysial antibody test, similar results in the two antibody tests, both positive or both negative, were obtained in 95% of the 466 relatives. The tissue transglutaminase antibody test found all but three low-titre endomysial antibody-positive family members. Additionally the

IgA- class tissue transglutaminase antibody test found 19 endomysial antibody negative cases. The overall correlation between the two tests was good (Spearman´s rank correlation coefficient 0.49, $p<0.0001$).

HLA typing was carried out on 52 out of 322 previously diagnosed coeliac disease or dermatitis herpetiformis patients; 51 of these were positive for HLA-DR3-DQ2 and one for HLA DR4-DQ8. (Figure 6)

All 44 endomysial antibody-positive but only 35 out of 48 gliadin antibody-positive individuals were positive for HLA DR3-DQ2.

Fourty-two (58%) of the antibody-positive individuals underwent small-bowel biopsies and 29 new coeliac disease patients were diagnosed (6.2% of 466 healthy family members). Endomysial antibodies detected 97% and gliadin antibodies 52% of the new cases. All newly diagnosed coeliac disease patients were positive for HLA-DR3-DQ2.

Figure 5. Study protocol and results of study **I**. CD = coeliac disease, EMA = endomysial antibodies, tTG = tissue transglutaminase antibodies, HLA = human leukocyte antigen.

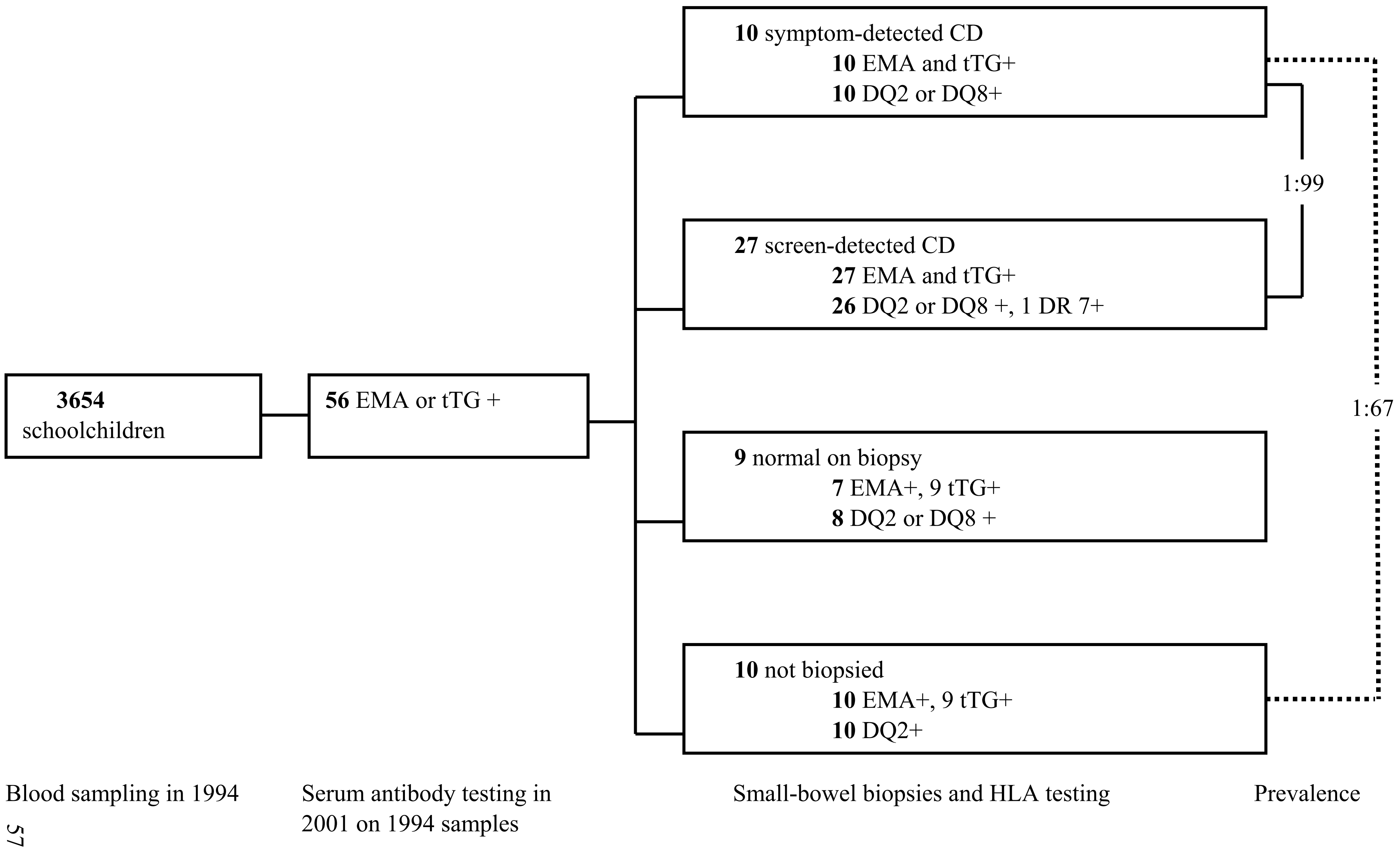
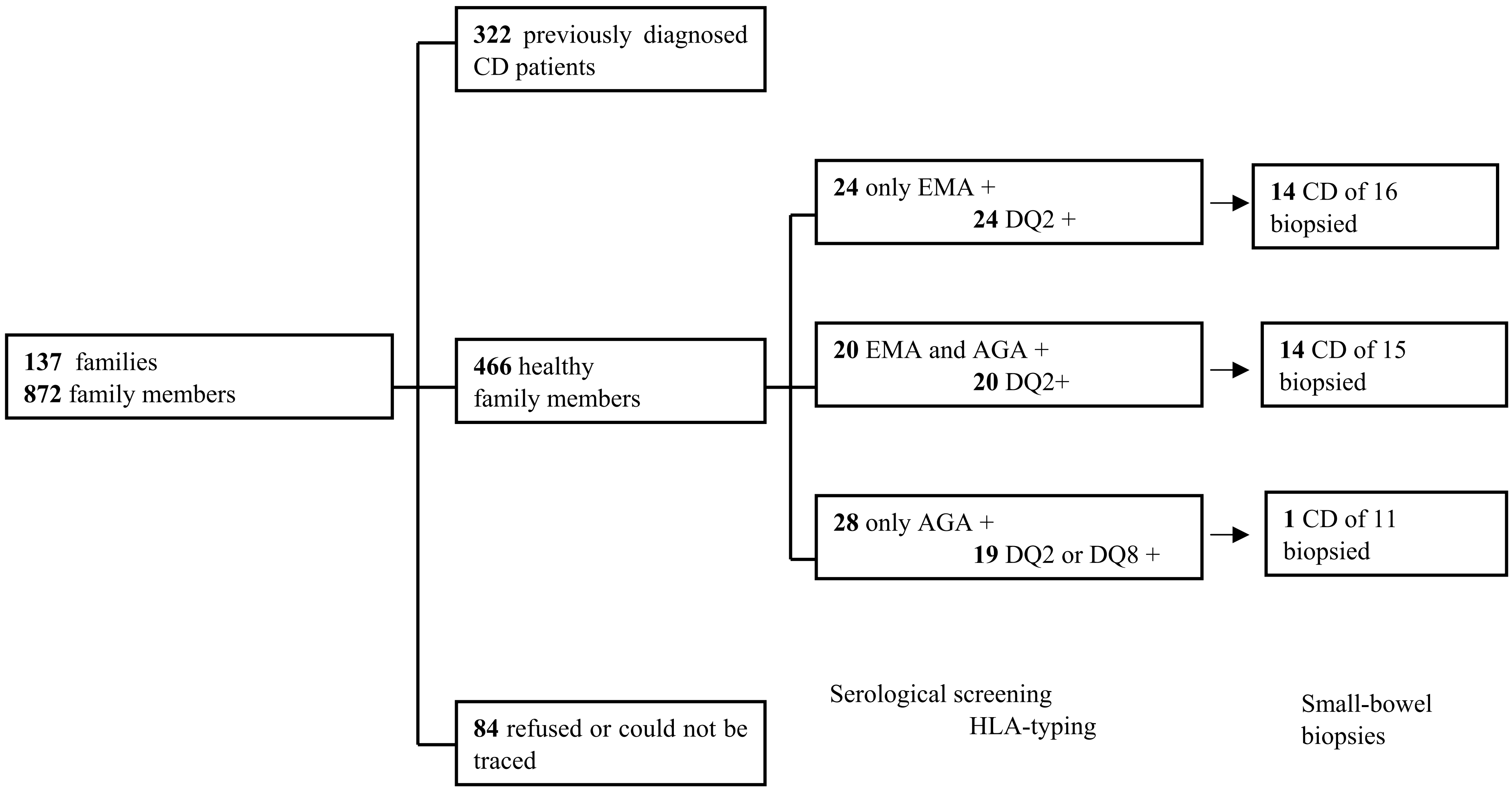


Figure 6. Study protocol and results of study **II**. CD = coeliac disease, EMA = endomysial antibodies, AGA = gliadin antibodies, HLA = human leukocyte antigen.



*Prevalence of different forms of coeliac disease (**I**)*

The number of biopsy-proven coeliac disease cases among the 3654 schoolchildren was 37 giving a minimum prevalence 1:99 (95% CI 1:146 – 1:75). Further 18 individuals were positive for coeliac disease- specific autoantibodies and carried HLA haplotypes associated with coeliac disease. If these individuals with genetic gluten intolerance are taken into account the overall prevalence of the coeliac trait among this population was 1:67 (95% CI 1:89 – 1:52).

Ten (27%) out of 36 subjects who filled in a questionnaire concerning symptoms related to coeliac disease reported no symptoms and they did not belong to the coeliac disease risk groups. The most common symptom reported was recurrent abdominal pain and intermittent diarrhoeal symptoms or constipation (11 out of 36). Four out of 36 subjects were first-degree relatives of coeliac disease patients.

Genetic background

*Coeliac disease risk factors among unselected population (**I**)*

Among 3627 schoolchildren who were HLA-typed, altogether 655 (18%) individuals carried the HLA DR3-DQ2 haplotype. Eighty of these (2.2% of the whole population) carried the combination HLA DR3-DQ2/DR4-DQ8; 756 (20.8% of wholw population) carried HLA DR4-DQ8 haplotype. Thus, altogether 1411 (39%) individuals among the whole school population carried haplotypes associated with coeliac disease. The most potent risk factor for developing endomysial or tissue transglutaminase autoantibodies was the HLA DR3-DQ2 haplotype (Odds ratio 26.45, PPV 8.00 %), the next most potent combination HLA DR3-DQ2/DR4-DQ8 (Odds ratio 1.66, PPV 2.50 %). The HLA DR4-DQ8 haplotype was the weakest risk factor (Odds ratio 0.45, PPV 0.79 %).

*Comparison between silent and symptomatic disease (**III**)*

All coeliac disease patients in study **III** carried at least one copy of the DQ2 heterodimer (i.e. the HLA DR3-DQ2 haplotype) regardless of the form of the disease. The distribution of HLA DR-DQ haplotypes in the two patient groups was similar and is shown in Table 7.

There were no statistically significant differences (Fisher´s exact test) in HLA-DR-DQ distributions between the sibs with silent coeliac disease and sibs with the symptomatic form. In fourteen (50%) of the 28 pairs the siblings shared both DR-DQ haplotypes identical by state.

Of all patients 25% were HLA DR3-DQ2 homozygous. There was no difference in the number of homozygous individuals between silent (n=6) and symptomatic (n=7) coeliac disease patients. In the group of silent patients 12% (2 out of 17) of women and 36% (4 out of 11) of men were homozygous for DQ2. In the symptomatic group the figures were 24% (5 out of 21) in women and 29% (2 out of 7) in men, respectively.

Table 7. Distribution of HLA-DR-DQ haplotypes in patients with the clinically silent and symptomatic forms of coeliac disease.

HLA-DR-DQ haplotypes	Clinically silent patients (n=28)	Symptomatic patients (n=28)
DR3-DQ2 homozygotes	6 (21.4%)	7 (25.0%)
DR3-DQ2 / DR7-DQ2	2 (7.2%)	2 (7.2%)
DR3-DQ2 / DR8-DQ4	4 (14.3%)	1 (3.5%)
DR3-DQ2 / DR1-DQ5	1 (3.5%)	5 (17.9%)
DR3-DQ2 / DR14-DQ5	-	1 (3.5%)
DR3-DQ2 / DR2-DQ6	7 (25.0%)	4 (14.3%)
DR3-DQ2 / DR6-DQ6	5 (17.9%)	5 (17.9%)
DR3-DQ2 / DR6-DQ7	1 (3.5 %)	1 (3.5%)
DR3-DQ2/DR4-DQ8	2 (7.2%)	2 (7.2%)

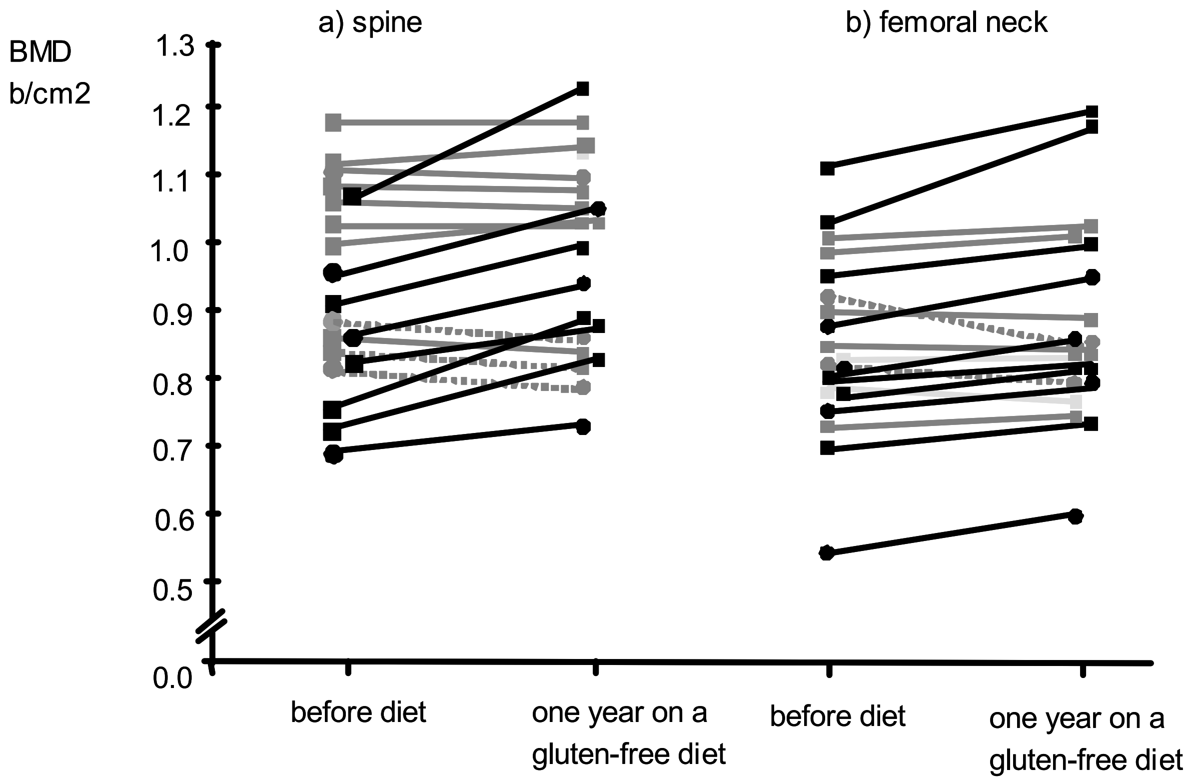
There was a statistically significant difference (p=0.037) in the median ages at time of diagnosis between the two groups of patients: the median age of clinically silent patients was 38 years (range 7-60) and that of symptomatic patients 29 years (range 8-51) at the time of diagnosis. In the group of symptomatic patients the HLA DR3-DQ2 homozygous individuals were diagnosed for the disease earlier (median age 19 years, range 12-42) than the heterozygous ones (median age 33 years, range 8-51), even though the difference was not statistically significant. Homozygocity for HLA DR3-DQ2 did not affect the time of onset of the disease in the silent coeliac disease group.

*Bone mineral density (**IV**)*

At the time of diagnosis the median t-scores of silent coeliac disease patients were –1.9 (95% CI from –2.4 to –1.4) in the spine and –0.9 (95% CI from –1.5 to –0.7) in the femoral neck. In symptomatic coeliac disease patients the median t-score values were –1.1 (95% CI from –1.5 to –0.7) and –0.8 (95% CI from –1.1 to –0.5), respectively. The bone mineral density of silent coeliac disease patients was in the spine even lower than that of symptomatic patients.

During one year's follow-up the spinal bone mineral density increased significantly in eight out of 19 clinically silent coeliac disease patients and that in the femoral neck in ten patients (Figure 7). The two individuals whose bone mineral density decreased significantly during one year follow-up did not concent to diet.

Figure 7. Bone mineral densities in 19 silent coeliac disease patients before gluten-free diet and after one year on a diet. ● indicates female and ■ male patients. Black lines show a significant increase and dotted lines a significant decrease in bone mineral density (BMD). An individual change was considered significant if its magnitude exceeded 2√2 times the short-term *in vivo* precision of the given measurement. Grey lines were used when there was no change in bone mineral density



Quality of life (V)

Among 19 screen-detected silent coeliac disease patients in study **V** there were 14 individuals who reported no signs or symptoms suggesting the probability of coeliac disease. Three had previously been diagnosed for lactose intolerance, one of them also for mild iron deficiency anaemia. Two patients had experienced obscure arthralgias.

Among 21 clinically diagnosed symptomatic coeliac disease patients there were 16 individuals suffering from abdominal symptoms; two had stomatitis, one was suffering from neurological symptoms and one had skin symptoms.

All coeliac disease patients were on a gluten-containing diet before the diagnosis (gluten consumption on average 13.2 g/day for silent and 10.2 g/day for symptomatic patients).

The changes in PGWB (Psychological General Well-Being) and GSRS (Gastrointestinal Rating Scale) during the one-year gluten-free diet treatment are shown in Figure 8.

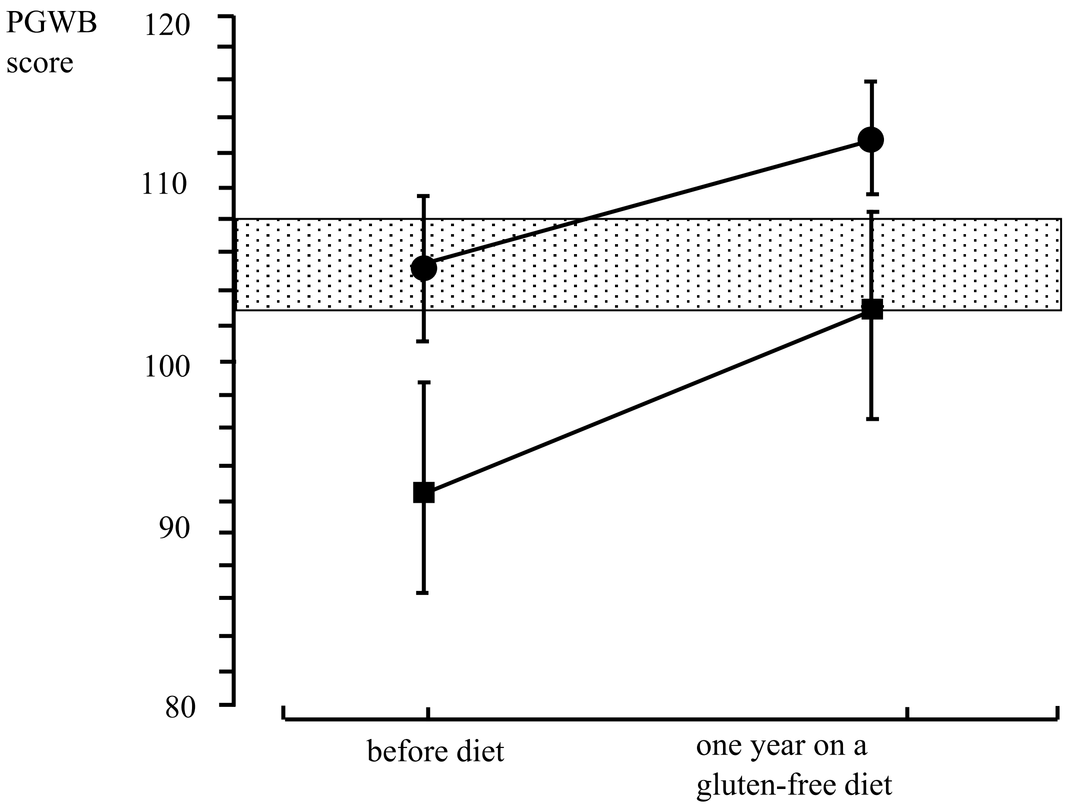
At the time of diagnosis the mean total PGWB of silent coeliac disease patients was within the same limits as that of non-coeliac controls but was significantly better ($p=0.001$) than the quality of life of symptomatic patients. The mean total PGWB of both silent and symptomatic coeliac disease patients improved significantly ($p<0.001$) during the gluten-free diet. The symptomatic coeliac disease patients achieved the level of non-coeliac controls, whereas the silent coeliac disease patients showed even higher scores than the controls.

For GSRS the trend was analogous to PGWB. At diagnosis the mean total GSRS value of silent coeliac disease patients was on a level with that of non-coeliac controls. In contrast, the symptomatic coeliac disease patients showed significantly ($p=0.004$) higher GSRS values than the silent patients or controls revealing that at the time of diagnosis the symptomatic patients really experienced more gastrointestinal symptoms than the silent disease subjects. The gluten-free diet significantly alleviated ($p=0.002$) gastrointestinal symptoms in both silent and symptomatic coeliac disease patients. After one year on a gluten-free diet the mean total GSRS score among symptomatic patients was at the same level as that of non-coeliac controls, while the scores of silent coeliac disease patients were even lower.

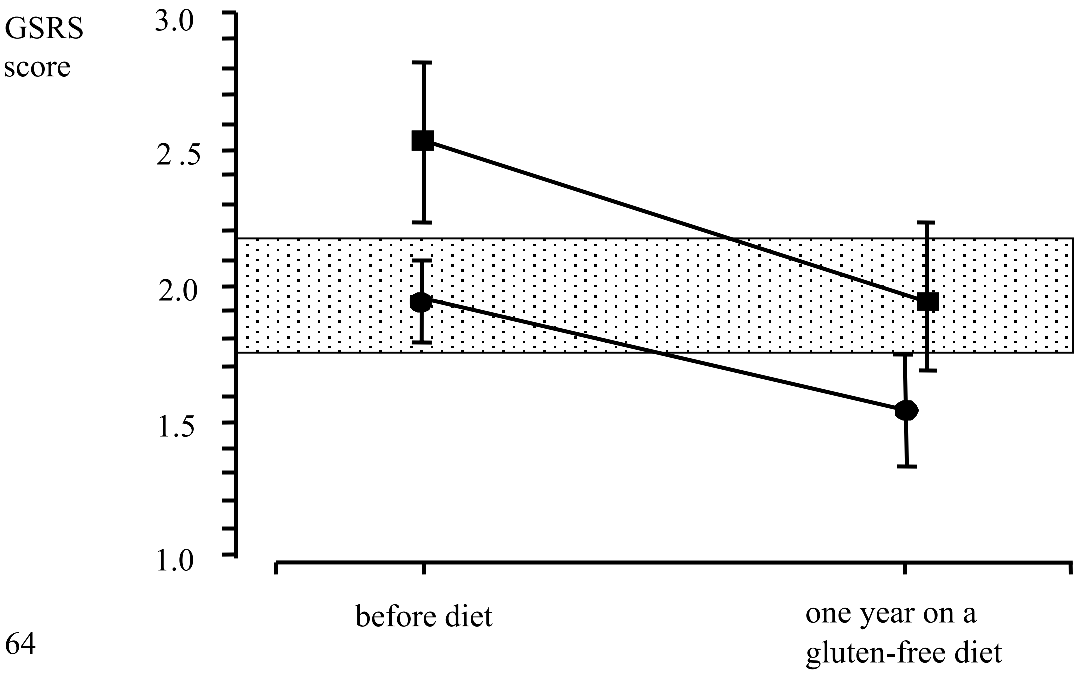
The self-estimated long-term adherence to the gluten-free diet on a visual analog scale was good (mean 95% for silent and 93% for symptomatic patients). Further, no dietary transgressions were observed in the four-day food records of seven silent or nine symptomatic coeliac disease patients.

Figure 8. Median psychological general well-being (PGWB) score (a) and median gastrointestinal rating scale (GSRS) score (b) with 95% confidence intervals among silent (●) and symptomatic (■) coeliac disease patients during the time of diagnosis and after one year on a gluten-free diet. The shaded area represents 95% confidence intervals for PGWB and GSRS scores of non-coeliac controls. Higher scores represent better quality of life.

a)



b)



DISCUSSION

Prevalence of coeliac disease

The present study showed the prevalence of coeliac disease found by serological screening to be higher than expected on the basis of clinical suspicion of the disease. Serological screening among an unselected cohort of schoolchildren revealed a biopsy-proven prevalence of coeliac disease of 1:99. This is in agreement with previous population-based screening studies among the Scandianvian population (Kolho et al. 1998, Hovdenak et al. 1999, Ivarsson et al. 1999a, Carlsson et al. 2001). It is to be noted that one third of the screening-detected cases reported no symptoms or signs compatible with coeliac disease or did not belong to risk groups of the disease. Thus, patients with silent coeliac disease constitute an important proportion of all coeliac disease patients on a population level.

The same phenomenon was observed among the material of multiple-case coeliac families originally collected for genetic studies: 6% of healthy family members were suffering from undiagnosed coeliac disease despite awareness of the clinical picture of the disorder and the high index of suspicion of the disease. Similar results have also been obtained in other studies among first-degree relatives of both coeliac disease (Korponay-Szabo et al. 1998, Petaros et al. 2002) and dermatitis herpetiformis patients (Hervonen et al. 2002). Thus, it is obvious that even in coeliac disease families the disease may remain undetected without active serological screening.

Interestingly, the prevalence figure obtained by population-based screening among unselected schoolchildren (1:99) did not diverge markedly from the figure obtained from the screening of an adult population (1:97) consisting of staff at Helsinki University Hospital (Kolho et al. 1998). This was also the case in Swedish studies: serological screening among healthy 2.5-year-old children revealed a prevalence figure as high as 1:100 (Carlsson et al. 2001), while among the adult population the prevalence figure was at the same level, 1:188 (Ivarsson et al. 1999a). These findings may support the conception that the disease could already be detected in childhood if coeliac disease-specific autoantibody screening were used systematically.

The value of antibody tests

The value of endomysial antibodies as a screening tool for coeliac disease was shown in both of our screening studies - among an unselected population of schoolchildren as well as among individuals with a high risk of the disease.

Of 54 endomysial antibody-positive individuals detected in the population of 3654 schoolchildren, 46 agreed to a small-bowel biopsy and 37 (80.4%) of them were found to have small-bowel mucosal lesion typical of coeliac disease. Further, it was noted that all endomysial antibody-positive individuals were carrying HLA haplotypes typical of coeliac disease. Results were similar in serological screening of first-degree relatives of coeliac disease patients: 44 individuals among 466 healthy family members were endomysial antibody-positive and in 28 (90.3%) out of 31 subjects who consented to small-bowel biopsy the diagnosis of coeliac disease was confirmed by the biopsy specimen. Also in this material all endomysial antibody-positive individuals were positive for HLA haplotypes associated with coeliac disease.

Judging by these findings it is obvious that in addition to good correlation with small-bowel mucosal findings of coeliac disease, endomysial antibodies stand also in very strong correlation with the coeliac type HLA haplotypes. These findings are supported by many authors (Cataldo et al. 1995, Hin et al. 1999, Carroccio et al. 2002, Kaukinen et al. 2002b), who have also found a good correlation between endomysial antibodies, small-bowel mucosal architecture and HLA-haplotypes associated with coeliac disease.

There are, however, opinions to the contrary (Dickey et al. 2000, Rostami et al. 2000). These authors claim that coeliac disease-specific findings in small-bowel mucosal specimens are also common in endomysial antibody- negative individuals. In their opinion the prevalence of coeliac disease is underestimated if endomysial antibodies are used as a criterion for small-bowel biopsies.

One explanation for the controversial results of different studies may be the fact that the timing of the appearance of endomysial antibodies and manifest mucosal lesion may be different. The endomysial antibodies may exist years prior to manifest mucosal lesion, but they may also turn out to be negative, since in a small minority of coeliac disease patients the endomysial antibody titres may fluctuate during the course of time.

In our schoolchildren population there were five carriers HLA-haplotypes associated with coeliac disease who evinced positive endomysial antibodies in 1994 but were negative for the antibodies in 2001. The small-bovel mucosal architecture was normal, but there were signs of mucosal inflammation in the biopsy specimen. One might speculate that the reason for this fluctuation could be a low intake of gluten: small amounts of gluten could keep the inflammation going on but would not affect the mucosal architecture. In our cases the dietitian was not able to register any dietary limitations to gluten consumption in these patients. In contrast we think that these five individuals represent a minority of coeliac disease patients with a fluctuating natural history of gluten sensitivity: a phenomenon which has previously been described in one paper from Kutlu and associates (Kutlu et al. 1993).

Further, our material included some endomysial antibody-positive individuals who evinced normal small-bowel mucosal architecture. Many previous studies have shown that the finding of normal mucosal architecture on small-bowel biopsy in an endomysial antibody-positive individual ingesting normal amounts of gluten does not always exclude coeliac disease; the villous atrophy and crypt hyperplasia may develop later in life (Mäki et al. 1990, Corazza et al. 1992, Collin et al. 1993, Troncone 1995). In fact, individuals with normal small-bowel mucosal architecture in our study already had some signs of mucosal inflammation in their gut. This has also been shown by Kaukinen and associates (Kaukinen et al. 2001): individuals without manifest mucosal lesion had increased numbers of intraepithelial lymphocytes in their gut as a sign of ongoing inflammation. Interestingly, the inflammation resolved and the gastrointestinal symptoms were alleviated when these individuals were prescribed a gluten-free diet. It is, thus, obvious that follow-up of these subjects is indicated, but debate continues as to whether they should be treated.

In many studies step-wise screening has been used: gliadin antibodies as the first step of screening and endomysial antibodies as the second step (Not et al. 1998, Meloni et al. 1999a, Gomez et al. 2001). Our study among first-degree relatives of coeliac disease patients showed that about one half of new coeliac disease cases would have remained undetected if gliadin antibodies alone had been used in the first-step evaluation. Further, only 15 out of 28 gliadin antibody- positive individuals showed villous atrophy and crypt hyperpasia in their small-bowel specimen, which indicates that positive gliadin antibodies do not correlate well with the biopsy findings. The correlation between gliadin antibodies and coeliac type HLA-DQ haplotypes was poor; 20% of gliadin antibody positive individuals were negative for HLA-DQ2 and –DQ8 and were thus probably truly wrong positives as to coeliac disease. These findings are in accord with those in other studies (Bardella et al. 2001, Lagerqvist et al. 2001) and on this bases we would infer that endomysial antibodies alone offer a suitable method for screening coeliac disease.

One problem with the endomysial antibody test is the subjectivity of interpretation of the indirect immunofluorescence test and thus the laboratory dependence of the test. In Europe endomysial antibody testing has been standardized and the K values for interlaboratory reproducibility are good, but without robust protocols and reference materials for standardization the test may still be unreliable (Stern 2000).

Since the finding that tissue transglutaminase is an endomysial autoantigen (Dieterich et al. 1997), several new ELISA tests for tissue transglutaminase antibodies have been developed by a number of manufacturers. The advantage of tissue transglutaminase antibody testing is that many commercial kits are available for detecting these antibodies and these testing methods are not dependent on the observer or the laboratory.

In this study we evaluated 466 first-degree relatives of coeliac disease patients using a commercial FDA-approved guinea-pig tissue transglutaminase kit, and correlated the results with the endomysial antibody findings. The correlation was good: in 444 out of 466 cases the two tests were in agreement. However, there were slight variations between the results of tissue transglutaminase and endomysial antibody tests in favour of latter.

In screening of the schoolchildren population we used another commercial tissue transglutaminase kit based on human recombinant tissue transglutaminase. The new method was chosen since in the literature there was accumulating evidence about the superiority of human recombinant tissue transglutaminase based kits compared to guinea pig transglutaminase based ones. With the new human recombinant tissue transglutaminase based method tissue transglutaminase antibodies correlated almost perfectly with endomysial antibodies: in only three cases were the results in disagreement.

In the literature many authors have suggested that the results of the tissue transglutaminase antibody test are comparable with those of the endomysial antibody test (Dieterich et al. 1998, Troncone et al. 1999, Hansson et al. 2000, Sblattero et al. 2000, Burgin-Wolff et al. 2002, Trevisiol et al. 2002). Others, however, have found the tissue transglutaminase antibody test less specific for coeliac disease than endomysial antibodies (Biagi et al. 2001b, Lagerqvist et al. 2001, Weile et al. 2002, West et al. 2002). The different results of the studies may partly be explained by different substrates used in the tests. Wong and associates (2002) compared 13 guinea pig and human tissue transglutaminase ELISA kits and found that kits based on human recombinant tissue transglutaminase demonstrated superior performance (especially specificity) to kits based on guinea pig tissue transglutaminase. Nevertheless they also found differences between different human recombinant tissue transglutaminase antibody tests suggesting that it is not only the substrate but also the method itself which affects results. Further, an unsolved question is whether low positive tissue transglutaminase antibody results in endomysial antibody-negative individuals are in fact wrong positives. If these cases have coeliac-type HLA they might be latent cases in a very early stage of the disease developing endomysial antibodies later. If this is the case, the tissue transglutaminase antibodies might be even more potent tool in predicting the disease than endomysial antibodies.

Human recombinant tissue transglutaminase antibody kits are both inexpensive and laboratory-independent methods for detecting untreated coeliac disease. This test should be favoured in first-step screening for the disease. In unclear situations, i.e. when an individual has abundant symptoms despite negative tissue transglutaminase test results, endomysial antibodies should be determined. Combination of the two tests increases the sensitivity of the screening without detriment to the specificity. In ambiguous cases HLA typing might yield further information, especially if the small-bowel biopsy finding is unclear. Also IgA-deficiency should be kept in mind when using IgA-class endomysial or tissue transglutaminase antibodies. In the case of IgA-class tissue transglutaminase antibodies below detection limit, total IgA as well as IgG-class endomysial and tissue transglutaminase antibodies should be tested. In the future, it will be important to reconsider the diagnostic criteria for coeliac disease. It is obvious that the sensitivity of the small-bowel biopsy in detecting genetic gluten intolerance is poor and autoantibody tests might provide an effective tool for diagnostics. However, in view of the few coeliac disease patients negative for both tissue transglutaminase and endomysial antibodies, gastrointestinal endoscopy should be performed if gastrointestinal symptoms are in evidence.

Genetic gluten intolerance

The current diagnostic criteria for coeliac disease stipulate total or subtotal villous atrophy and crypt hyperplasia in the small-bowel mucosal biopsy specimen. In our population-based screening study there were several individuals who did not fulfil the current criteria for the disease, since they refused small-bowel biopsy or had normal small-bowel mucosal architecture in their biopsy specimen. This notwhithstanding, these individuals were positive for autoantibodies and HLA haplotypes associated with coeliac disease. According to the current criteria these individuals should be excluded from the prevalence figures. However, it is known from many previous studies that the genetically predisposed autoantibody-positive individuals with normal small-bowel mucosal architecture may develop the disease later in life (Collin et al. 1993, Holm 1993a, Troncone 1995). Moreover, it is known that complications related to coeliac disease may emerge already in the asymptomatic stage of the disease (Mazure et al. 1994, Collin et al. 1996, MacGowan et al. 1996, Hadjivassiliou et al. 1997, Kolho et al. 1999, Meloni et al. 1999b) or even when the small-bowel mucosal damage is not yet detectable (Calcani et al. 2001, Kaukinen et al. 2001). For these reasons the current criteria for the disease have been criticized. Genetic gluten intolerance is a new term introduced to describe individuals evincing coeliac disease-specific autoantibodies in their serum specimen and carrying an HLA haplotype compatible with coeliac disease. Judging from our material the overall prevalence of genetic gluten intolerance in the Finnish population is as high as 1:67.

Genetic background

The genetic background of coeliac disease is beyond doubt: in our multiple-case coeliac families as many as 40% of all family members were suffering from the disease. This results partly from our original precondition to include families with at least two affected cases because the material was initially collected for genetical studies. However, it is also possible that there are some special risk factors enriched among these families causing the high number of patients. To identify individuals running an increased risk of genetic gluten intolerance information on the genetic risk factors behind coeliac disease is of prime importance.

Risk factors

In this study the HLA DR3-DQ2 haplotype was identified as the most potent risk factor for coeliac disease: 86% of all endomysial antibody-positive individuals carried this haplotype in the unselected schoolchildren population. The Odds ratio for this haplotype being conductive to endomysial antibody-positivity was as high as 26.45. These findings confirm the data published in many countries: about 90% of coeliac disease patients carry the HLA DR3-DQ2 haplotype (Bugawan et al. 1989, Sollid & Thorsby 1990, Mazzilli et al. 1992, Congia et al. 1994, Fernandez et al. 1995, Howell et al. 1995, Polvi et al. 1996). Only people of Jewish descent seem to be an exception, having a lower proportion of coeliac disease patients carrying HLA DR3-DQ2 (Tighe et al. 1993).

The remaining endomysial antibody-positive individuals negative for the HLA DR3-DQ2 haplotype in the present study carried mainly the HLA DR4-DQ8 haplotype. One individual carried the DQB1*02 part of the DQ2 heterodimer. A similar phenomenon is to be observed in other populations: the HLA DR4-DQ8 haplotype seems to be an important risk factor behind coeliac disease besides the HLA DR3-DQ2 haplotype (Spurkland et al. 1992, Tighe et al. 1992, Tighe et al. 1993). However, in our study the Odds ratio for an individual carrying the HLA DR4-DQ8 but not HLA DR3-DQ2 haplotype to be positive for endomysial antibodies was only 0.45, i.e. much lower than that for the HLA DR3-DQ2 haplotype. It would thus appear that the HLA DR4-DQ8 haplotype is a much less prominent risk factor for coeliac disease than the HLA DR3-DQ2 haplotype. The low Odds ratio is probably dependent on the low number coeliac disease patients among all individuals carrying the HLA DR4-DQ8 haplotype: only 1% of them are suffering form coeliac disease whereas among HLA DR3-DQ2 positive individuals the proportion of coeliac disease patients is 6.5%. Interestingly, individuals heterozygotic for the two main risk factors (HLA DR3-DQ2 and HLA Dr4-DQ8) carried lower risk of antibody positivity than those carrying only HLA DR3-DQ2 (Odds ratio 1.66). This is a new finding which calls for further evaluation even though the proportion of individuals heterozygous for the two main risk factors is only 2% of all coeliac disease patients.

It should be recalled here that on the population level the coeliac disease-related HLA haplotypes are more common than the disease itself. In our study altogether 1411 individuals (39% of the whole population) carried HLA-haplotypes connected with coeliac disease, but only 56 (4%) of them were positive for endomysial or tissue transglutaminase antibodies. It follows that the HLA risk factors do not of themselves induce the onset of the disease; other genetic or environmental factors must be involved.

However, the prevalence of the disease and the gene frequency of HLA DR3-DQ2 haplotype positivity seem to correlate with each other. According to the present study in our population the gene frequency is around 10%, whereas in Saharawi people the frequency is as high as 23% (Catassi et al. 2001). Population-based screening studies have shown that the prevalence of coeliac disease among Saharawi children is highest in the world (1:18) (Catassi et al. 1999). It would indeed appear that the gene frequency of HLA DR3-DQ2 varies in different countries (Lampis et al. 2000), this inevitably affecting the prevalence of coeliac disease in these countries (Table 8). In Japan and China the disease is almost unknown.

It may be speculated that the HLA DR3-DQ2 haplotype constituted during the course of time a protective factor for mankind and was therefore preserved through the generations. For hundreds of thousands of years the human being needed this HLA-factor to survive infectious diseases. Agriculture only developed about 10 000 years ago and was based mainly on non-gluten-containing cereals. Gluten was thus introduced to mankind only during the last hundreds of years of history. Concomitant with the increasing amount of gluten in daily intake and the improving nutritional status of individuals the number of infections has decreased (Keusch 2003). It may thus be hypothesized that in some genetically determined individuals the natural immunological process which was first targeted to infections must have chosen a new target, gluten, and acts in protecting the body from this agent, developing coeliac disease. This theory is supported by the case of the Saharawi people. These people were living in the deserts and their gluten consumption was low. After relocation in refugee camps, however, they began to ingest more gluten and genetically susceptible individuals started to develop coeliac disease. In many European countries gluten was introduced to the daily diet in earlier periods and natural selection among individuals may have affected the genetic background of these populations, with the result that the frequency of HLA DR3-DQ2 haplotype is decreasing and other genetic or environmental factors are affecting the onset and the clinical picture of the disease. It would thus be understandable, that mild forms of the disease are becoming more frequent and that there are local differences in its prevalence.

Table 8. Prevalence of coeliac disease in unselected population-based studies and HLA DRB1*03, DQA1*05 and DQB1*02 gene frequencies in different countries.

Country	*HLA DRB1*03,DQA1*05,DQB1*02 gene frequency (%)*	*Reference*	*Prevalence*	*Reference*
Finland	9.0	Current study	1:99	Current study
Sweden	11.0	(Allen et al. 1994)	1:77	(Carlsson et al. 2001)
Norway	14.0	(Rönningen et al. 1990)	1:340	(Hovdenak et al. 1999) stepwise testing
Germany	7.7	(Imanishi 1992)	1:2272	(Henker & Tandler 1993)
UK	13.0	(Doherty et al. 1992)	1:122	(Johnston et al. 1998)
Spain	9.7	(Imanishi 1992)	1:389	(Riestra et al. 2000)
Italy	12.0	(Tighe et al. 1992)	1:204	(Volta et al. 2001a)
Saharawi	23.0	(Catassi et al. 2001)	1:18	(Catassi et al. 1999)
U.S.A. (White)	11.7	(Lampis et al. 2000)	1:250	(Not et al. 1998) stepwise testing
Japan	0	(Lampis et al. 2000)	-	No prevalence studies exist
China	3.2	(Lampis et al. 2000)	-	No prevalence studies exist

Effect of genetic factors on clinical picture

In addition to the prevalence figures, also the multidimensional clinical picture of coeliac disease may be determined by the genetic background. Some studies have suggested that the HLA DR3-DQ2 dosage affects or certain DQ combinations could determine the clinical outcome of coeliac disease (Ploski et al. 1993, Congia et al. 1994, Howell et al. 1995, Polvi et al. 1996). The studies in question have relied on the case-control setting, in which series of patients with different forms of the disease have been compared. In the present study we compared the HLA findings behind two different disease entities – silent and symptomatic forms of the disease – in a family-based approach. HLA haplotypes in siblings with different types of disease entities were compared to each other. No differences were noted in the HLA haplotypes of silent and symptomatic coeliac disease patients. These results are in agreement with the findings of Greco and associates (Greco et al. 1998b) who studied the correlation in a case-control setting. The only evidence of a DQ2 dosage effect was that in the group of silent coeliac disease patients women with this form of the disease seemed to be more often heterozygous for HLA-DQ2 than the men. Thus, a single dose of HLA-DQ2 could induce the onset of silent coeliac disease in women, whereas some additional factors may be required for its onset in men. This phenomenon has also been described by Holopainen and colleagues (Holopainen et al. 2001) who found evidence that HLA-DQ2 confers stronger susceptibility to coeliac disease in females as compared to males. Further, we found here that the age at onset was lower in clinically symptomatic than in silent cases. It was however not clear whether this was due to the DQ2 dosage effect, since the median age of silent coeliac disease patients at diagnosis is dependent on the time of screening.

The limitation of a family-based approach is the low number of subjects. The power of the study may not suffice to expose genetic differences between different disease entities. This is a problem especially in diseases inherited in a multifactorial manner. However, the family-based approach also has several advantages: the genetic background is homogeneous, the environmental factors are the same for the subjects compared to each other and most probably the diagnostic practice also is the same. Thus, the disadvantages of genetic heterogeneity, confounding environmental factors and different clinical practises can be avoided.

All in all, however, the findings of the present study make it clear that genetic risk factors outside HLA may play an important role in determining the clinical picture of the disease.

Benefits of the gluten-free diet

The benefits of a gluten-free diet in silent coeliac disease have been poorly investigated. A few studies suggest that early diagnosis and proper treatment of the disorder might protect patients from other autoimmune diseases (Pocecco & Ventura 1995, Ventura et al. 1999) but the matter remains controversial (Sategna-Guidetti et al. 2001). For the most part, however, the value of a gluten-free diet in sustaining health and preventing disease in silent coeliac disease remains to be demonstrated.

Osteopoenia

Bone mineral density has been observed to be diminished in both untreated silent and symptomatic coeliac disease patients (Corazza et al. 1995b, Kemppainen et al. 1999, Kalayci et al. 2001, Meyer et al. 2001). This was also seen to be the case in the present study: atthe time of diagnosis the median t-scores for silent coeliac disease patients were -1.9 in the spine and -0.9 in the femoral neck, and in symptomatic patients -1.1 and -0.8, respectively. Thus, both untreated silent and symptomatic coeliac disease patients showed osteopoenia in the spine and some of them also in the femoral neck. Since this decreased bone mineral density in coeliac disease patients may increase the number of fractures (Vasquez et al. 2000, Fickling et al. 2001), it is important to understand whether the problem can be solved by gluten-free diet treatment.

This study was the first to show that the bone mineral density of coeliac disease patients improves during a gluten-free diet even if the diagnosis was based on a serological screening. The spinal bone mineral density improved significantly in eight and the femoral bone mineral density in ten out of 19 silent coeliac disease patients during one year of gluten-free diet treatment. In the rest of the study population the bone mineral density remained unchanged except in two individuals who did not maintain a gluten-free diet. These individuals showed a significant decrease in bone mineral density during the follow-up. In accord with the present findings other authors have also found the gluten-free diet to be beneficial for the bone mineral density of coeliac disease patients (Mora et al. 1998, Kemppainen et al. 1999, Sategna-Guidetti et al. 2000, Kalayci et al. 2001). Thus, gluten-free diet treatment would indeed appear to be beneficial for the bone mineral density of coeliac disease patients, but prospective studies are lacking as to whether early diagnosis of the disease and early introduction of a gluten-free diet can also prevent fractures in coeliac disease patients.

Quality of life

As a chronic disorder coeliac disease may influence the quality of life of patients in many ways. For some a gluten-free diet may be a relief, since patients are often very well aware of the complication associated with untreated coeliac disease, but on the other hand the burden of life-long diet treatment may be heavy. In this study we evaluated the effect of gluten-free diet to the quality of life of adult coeliac disease patients using Psychological General Well Being questionnaire which has been thoroughly validated and has in many studies proved a reliable tool in detecting variation in the quality of life in different diseases (Dahlöf & Dimenäs 1994, Glia & Lindberg 1997, Havelund et al. 1999). The gastrointestinal symptoms were evaluated by using Gastrointestinal Rating Scale questionnaire which has been tested in many clinical trials (Dimenäs et al. 1995, Blomqvist et al. 1996, Revicki et al. 1998) and has proved reliable (Dimenäs et al. 1996). Results of the study groups were compared to those of a control group representing non-coeliac population. The confounding factors (age, sex, economic situation and body mass index) did not affect the results and the groups were comparable to each other even though the control group was larger than the study groups.

Gluten-free diet treatment seemed beneficial from the quality of life point of view. The quality of life of both silent and symptomatic coeliac disease patients seemed to improve during one year's follow-up on a gluten-free diet. At the same time the severity of gastrointestinal symptoms seemed to decrease in both symptomatic, and in screen-detected coeliac disease patients. In this study we used the distribution-based interpretation (Lydick & Epstein 1993) comparing the study results to the means of a particular non-coeliac reference population. This comparison revealed that after one year's follow-up the silent patients apparently felt even better than the non-coeliac controls. The implications of these findings may be variously envisaged.

First of all it is possible that the study itself affects the quality of life, since individuals joining the study may be influenced by the investigators. This possibility was here minimized by using self-administered questionnaires delivered to the patients by mail. Otherwise the subjects were treated according to a routine practice.

Further, it may be asked, whether the screen-detected coeliac disease patients were truly symptomless at the time of diagnosis. It should be emphasized here that both the psychological general well-being as well as the gastrointestinal symptoms of silent coeliac disease patients were on a level with non-coeliac patients at the time of diagnosis. However, when specifically asked, five individuals among the screen-detected coeliac disease patients acknowledged minor symptoms consistent with coeliac disease. These individuals experienced the gluten-free diet treatment as most beneficial. These findings imply that even individuals with silent coeliac disease may have minor symptoms of which they become aware only after being prescribed a gluten-free diet.

One particularly important question is whether the follow-up time was long enough to reveal real changes in the quality of life. It is questionable whether the benefit obtained from a gluten-free diet is permanent. Hallert and associates (Hallert et al. 1998) studied adult coeliac disease patients after ten years on a gluten-free diet and observed that patients failed to attain the same level of well-being as that of the general population. Especially women perceive the disease burden to be heavy (Hallert et al. 2002). In contrast, children placed on a gluten-free diet because of coeliac disease reported a quality of life similar to that of the reference sample (Kolsteren et al. 2001). For the most part the impaired quality of life is explained by poor compliance of adults during the course of time. Fabiani and colleagues (Fabiani et al. 2000) have suggested that long-term dietary adherence is poor especially among silent coeliac disease patients. However, a recent case-control study (Usai et al. 2002) has shown that the quality of life is not impaired only because of poor compliance but also by a variety of circumstances such as comorbidity. Adequate counselling and patient encouragement may be crucial in attaining a permanent improvement in the quality of life.

SUMMARY AND CONCLUSIONS

This study revealed beyond doubt that silent coeliac disease is an important disease entity which is often underestimated. One third of the patients detected by a population-based screening may be totally symptomless. Even among multiple- case coeliac families the disease may remain undiagnosed without active serological screening despite awareness of the disease. Endomysial and tissue transglutaminase antibodies correlate well with each other, with small-bowel mucosal findings of coeliac disease and with a coeliac disease-correlated genetic background. These coeliac disease-specific autoantibodies thus provide a reliable means of detecting the silent form of the disease. Interestingly, the disease-specific autoantibodies can be detected in the blood years before the manifest disease – in many cases already in childhood.

The prevalence of biopsy-proven coeliac disease among the Finnish population is as high as 1:99 and that of genetic gluten intolerance is 1:67. The major genetic risk factors there are the HLA DR3-DQ2 and HLA DR4-DQ8 haplotypes. However, differences in HLA haplotypes do not explain the different clinical outcomes of the disease: other genetic markers outside the HLA area as well as the impact of environmental factors should also be considered.

Even though silent coeliac disease is commonly found both among risk groups and in the normal population, population-based screening for the disease is not acceptable before the benefits of early diagnosis and treatment have been thoroughly evaluated. In this study we were able to show that the bone mineral density of silent coeliac disease patients is decreased at the time of diagnosis but increases during gluten-free diet treatment. This does not necessarily mean that the number of fractures could also be reduced by means of a gluten-free diet.

According to the present findings a gluten-free diet is at least over the short term acceptable and may even provide quality-of-life benefit to patients with silent coeliac disease. Impairment of the quality of life is thus no obstacle to screening for the disease.

Nonetheless, more evidence regarding the long-term benefits (and disadvantages) of early diagnosis and treatment of silent coeliac disease is needed and cost-benefit calculations should be undertaken before population-based screening programmes for coeliac disease can be suggested. Meanwhile, case-finding by screening is recommended.

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REFERENCES

Aitken RC (1969): Measurement of feelings using visual analogy scales. Proc R Soc Med 62:993-996

Allen M, M. S-W, Sjögren K, Erlich HA, Petterson U and Gyllensten U (1994): Association of susceptibility to multiple sclerosis in Sweden with HLA class II DRB1 and DQB1 alleles. Hum Immunol 39:41-48

Arentz-Hansen EH, Mc Adam SN, Molberg O, Kristianssen C and Sollid LM (2000): Production of a panel of recombinant gliadins for the characterisation of T cell reactivity in coeliac disease. Gut 46:46-51

Arvola T, Mustalahti K, Saha MT, Vehmainen P, Partanen J and Ashorn M (2002): Celiac disease, thyrotoxicosis, and autoimmune hepatitis in a child. J Pediatr Gastroenterol Nutr 35:90-92

Ascher H, Lanner A and Kristiansson B (1990): A new laboratory kit for anti-gliadin IgA at diagnosis and follow-up of childhood celiac disease. J Pediatr Gastroenterol Nutr 10:443-450

Ascher H, Hahn Z-M, Hanson LA, Kilander AF, Nilsson LA and Tlaskalova H (1996): Value of serologic markers for clinical diagnosis and population studies of coeliac disease. Scand-J-Gastroenterol 31:61-67

Askling J, Linet M, Gridley G, Halstensen TS, Ekström K and Ekbom A (2002): Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. Gastroenterology 123:1428-1435

Bardella MT, Marino R, Barbareschi M, Bianchi F, Faglia G and Bianchi P (2000): Alopecia areata and coeliac disease: no effect of a gluten.free diet on hair growth. Dermatology 200:108-110

Bardella MT, Trovato C, Cesana BM, Pagliari C, Gebbia C and Perrachi M (2001): Serological markers for coeliac disease: is it time to change? Dig Liver Dis 33:426-431

Bernasconi A, Bernasconi N, Andermann F, Dubeau F, Guberman A, Gobbi G, Olivier A (1998): Celiac disease, bilateral occipital calcifications and intractable epilepsy: mechanisms of seizure origin. Epilepsia 39:300-306

Bevan S, Popat S, Braegger CP, Busch A, O'Donahue D, Falth. Magnusson K, Ferguson A, Godkin A, Hogberg L, Holmes G, Hosie KB, Howdle PD, Jenkins H, Jewell D, Johnston S, Kennedy NP, Kerr G, Kumar P, Logan RF, Love AH, Marsh M, Mulder CJ, Sjöberg K, Stenhammer L and Houlston RS (1999): Contribution of the MHC region to the familial risk of coeliac disease. J Med Genet 36:687-690

Biagi F, Pezzimenti D, Campanella J, Vadacca GB and Gorazza GR (2001): Endomysial and tissue transglutaminase antibodies in coeliac sera: a comparison not influenced by previous testing. Scand J Gastroenterol 36:955-958

Blomqvist A, Lönroths H, Dalenbäck J, Ruth M, Wiklund I and Lundell L (1996): Quality of life assessment after laparoscopic and open fundoplications:results of a prospective, clinical study. Scand J Gastroenterol 31:1052-1058

Bonamico M, Mariani P, Danesi HM, Crisogianni M, Failla P, Gemme G, Quartino AR, Giannotti A, Castro M, Balli F, Lecora M, Andria G, Guariso G, Gabrielli O, Catassi C, Lazzari R, Balocco NA, De Virgiliis S, Culasso F and Romano C (2001a): Prevalence and clinical picture of celiac disease in Italian Down syndrome patients: a multicenter study. J Pediatr Gastroenterol Nutr 33:139-143

Bonamico M, Tiberti C, Picarelli A, Mariani P, Rossi D, Cipolletta E, Greco M, Tola MD, Sabbatella L, Carabba B, Magliocca FM, Strisciuglio P and Di Mario U (2001b): Radioimmunoassay to detect antitransglutaminase autoantiboides is the most sensitive and specific screening method for celiac disease. Am J Gastroenterol 96:1536-1540

Bottaro G, Volta U, Spina M, Rotolo N, Sciacca A and Musumeci S (1997): Antibody pattern in childhood celiac disease. J Pediatr Gastroenterol Nutr 24:559-562

Bowling A (1995): Measuring disease. Review of disease-specific quality of life measurement scales. Philadelphia: Open Univ Pr:14-16

Brandtzaeg P, Halstensen TS, Kett K, Krajci P, Kvale D, Rognum TO, Scott H and Sollid LM (1989): Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. Gastroenterology 97:1562-1584

Bugawan TL, Angelini G, Larrick J, Auricchio S, Ferrara GB and Erlich HA (1989): A combination of a particular HLA-DP beta allele and an HLA-DQ heterodimer confers susceptibility to coeliac disease. Nature 339:470-473

Bulpitt CJ, Dollery CT and Carrie S (1974): A symptom questionnaire for hypertensive patients. J Chron Dis 27:309-323

Burgin-Wolff A, Dahlbom I, Hadziselimovic F and Petersson C (2002): Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. Scand J Gastroenterol 37:685-691

Cacciari E, Salardi S, Lazzari R, Cicognani A, Collina A, Pirazzoli P, Tassoni P, Biasco G, Corazza GR and Cassio A (1983): Short stature and celiac disease: a relationship to consider even in patients with no gastrointestinal tract symptoms. J Pediatr 103:708-711

Calcani MJ, Parisi P, Guaitolini C, Parisi G and Paolone G (2001): Latent coeliac disease in a child with epilepsy, cerebral calcifications, drug-induced systemic lupus erythematosus and intestinal folic acid malabsortion associated with impairment of folic acid transport across the blood-brain barrier. Eur J Pediatr 160:288-292

Cambel H and Braidwood RJ (1970): An old farmer's village in Turkey. Le Scienze 22:96-103

Cammarota G, Fedeli G, Tursi A, Corazza GR and Gasbarrini G (1996): Coeliac disease and follicular gastritis. Lancet 347:268

Carlsson A, Axelsson I, Borulf S, Bredberg A, Forslund M, Lindberg B, Sjöberg K and Ivarsson SA (1998): Prevalence of IgA-antigliadin antibodies and IgA-antiendomysium antibodies related to celiac disease in children with Down syndrome. Pediatrics 101:272-275

Carlsson AK, Axelsson IE, Borulf SK, Bredberg AC, Lindberg BA, Sjöberg KG and Ivarsson SA (1999): Prevalence of IgA-antiendomysium and IgA-antigliadin autoantibodies at diagnosis of insulin-dependent diabetes mellitus in Swedish children and adolescents. Pediatrics 103:1248-1252

Carlsson AK, Axelsson IE, Borulf SK, Bredberg AC and Ivarsson SA (2001): Serological screening for celiac disease in healthy 2.5-year-old children in Sweden. Pediatrics 107:42-45

Carroccio A, Vitale G, Di Prima L, Chifari N, Napoli S, La Russa C, Gulotta G, Averna MR, Montalto G, Mansueto S and Notarbartolo A (2002): Comparison of anti-transglutaminase ELISAs and an anti-endomysial antibody assay in the diagnosis of celiac disease: a prospective study. Clin Chem 48:1546-1550

Castellino F, Scaglione N, Grosso SB and Sategna-Guidetti C (2000): A novel method for detecting IgA endomysial antibodies by using human umbilical vein endothelial cells. Eur J Gastroenterol Hepatol 12:45-49

Cataldo F, Ventura A, Lazzari R, Balli F, Nassimbeni G and Marino V (1995): Antiendomysium antibodies and coeliac disease: solved and unsolved questions. An Italian multicentre study. Acta Paediatr 84:1125-1131

Cataldo F, Marino V, Bottaro G, Greco P and Ventura A (1997): Celiac disease and selective immunoglobulin A deficiency. J Pediatr 131:306-308

Cataldo F, Marino V, Ventura A, Bottaro G and Corrazza GR (1998): Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP) and "Club det Tenue" Working Groups on Coeliac disease. Gut 42:362-365

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

Catassi C, Ratsch IM, Gandolfi L, Pratesi R, Fabiani E, El Asmar R, Frijia M, Bearzi I and Vizzoni L (1999): Why is coeliac disease endemic in the people of the Sahara? Lancet 354:647-648

Catassi C, Doloretta-Macis M, Ratsch IM, De Virgiliis S and Cucca F (2001): The distribution of DQ genes in the Saharawi population provides only a partial explanation for the high celiac disease prevalence. Tissue Antigens 58:402-406

Catassi C, Fabiani E, Corrao G, Barbato M, De Renzo A, Carella AM, Gabrielli A, Leoni P, Carroccio A, Baldassarre M, Bertolani P, Caramaschi P, Sozzi M, Guariso G, Volta U and Corrazza GR (2002): Risk of non-Hodgkin lymphoma in celiac disease. JAMA 287:1413-1419

Cellier C, Delabesse E, Helmer C, Patey N, Matuchansky C, Jabri B, Macintyre E, Cerf-Bensussan N and Brousse N (2000): Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. Lancet 356:178-179

Cerf-Bensussan N, Schneeberger EE and Bhan AK (1983): Immunohistologic and immunoelectron microscopic characterization of the mucosal lymphocytes of human small intestine by the use of monoclonal antibodies. J Immunol 130:2615-2622

Chorzelski TP, Sulej J, Tchorzewska H, Jablonska S, Beutner EH and Kumar V (1983): IgA class endomysium antibodies in dermatitis herpetiformis and coeliac disease. Ann N Y Acad Sci 420:325-334

Christiansen C, Rödbro P and Jensen H (1975): Bone mineral content in the forearm measured by photon absorptiometry. Principles and reliability. Scand J Clin Lab Invest 35:323-330

Ciclitira PJ, Ellis HJ and Evans DJ (1983): A solid-phase radioimmunoassay for measurement of circulating antibody titres to wheat gliadin and its subfractions in patients with adult coeliac disease. J Immunol Methods 62:231-239

Collin P, Pirttilä T, Nurmikko T, Somer H, Erilä T and Keyriläinen O (1991): Celiac disease, brain atrophy and dementia. Neurology 41:372-375

Collin P, Mäki M, Keyriläinen O, Hällström O, Reunala T and Pasternack A (1992): Selective IgA deficiency and coeliac disease. Scand J Gastroenterol 27:367-371

Collin P, Helin H, Mäki M, Hällström O and Karvonen A-L (1993): Follow-up of patients positive in reticulin and gliadin antibody tests with normal small bowel biopsy findings. Scand J Gastroenterol 28:595-598

Collin P, Mäki M, Rasmussen M, Kyrönpalo S, Pehkonen E and Reunala T (1995): The prevalence of adult coeliac disease in Finland. Scand J Gastroenterol 30,Suppl 209:99

Collin P, Vilska S, Heinonen PK, Hällström O and Pikkarainen P (1996): Infertility and coeliac disease. Gut 39:382-384

Collin P, Reunala T, Rasmussen M, Kyrönpalo S, Pehkonen E, Laippala P and Mäki M (1997): High incidence and prevalence of adult coeliac disease. Augmented diagnostic approach. Scand J Gastroenterol 32:1129-1133

Collin P, Kaukinen K, Välimäki M and Salmi J (2002): Endocrinological disorders and celiac disease. Endocr Rev 2002:464-483

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

Cook HB, Burt MJ, Collett JA, Whitehead MR, Frampton CM and Chapman BA (2000): Adult coeliac disease: prevalence and clinical significance. J Gastroenterol Hepatol 15:1032-1036

Cooke WT and Holmes GKT (1984): Coeliac disease. Churchill Livingstone Press, Edinburgh

Cooper BT, Holmes GK and Cooke WT (1982): Lymphoma risk in coeliac disease of later life. Digestion 23:89-92

Corazza GR, Tabacchi P, Frisoni M, Londei M, Bastia D and Gasbarrini G (1983): T-lymphocyte subsets in adult coeliac disease. Clin Sci 65:89-90

Corazza GR, Frazzoni M and Gasbarrini G (1984): Jejunal intraepithelial lymphocytes in coeliac disease: are they increased or decreased? Gut 25:158-162

Corazza G, Valentini RA, Frisoni M, Volta U, Corrao G, Bianchi FB and Gasbarrini G (1992): Gliadin immune reactivity is associated with overt and latent enteropathy in relatives of celiac patients. Gastroenterology 103:1517-1522

Corazza GR, Andreani ML, Venturo N, Bernardi M, Tosti A and Gasbarrini G (1995a): Celiac disease and alopecia areata: report of a new association. Gastroenterology 109:1333-1337

Corazza GR, Di Sario A, Cecchetti L, Tarozzi C, Corrao G, Bernardi M and Gasbarrini G (1995b): Bone mass and metabolism in patients with celiac disease. Gastroenterology 109:122-128

Cornell H, Wieser H and Belitz HD (1992): Characterization of the gliadin-derived peptides which are biologically active in coeliac disease. Clin Chim Acta 213:37-50

Corrao G, Corazza GR, Andreani ML, Torchio P, Valentini RA, Galatola G, Quaglino D, Gasbarrini G and di Orio F (1994): Serological screening of coeliac disease: choosing the optimal procedure according to various prevalence values. Gut 35:771-775

Csizmadia C, Mearin M, von Blomberg B, Brand R and Verloove-Vanhorick S (1999): An iceberg of childhood coeliac disease in the Netherlands. Lancet 353(9155):813-814

Cullum ID, Ell PJ and Ryder JP (1989): X-ray dual photon abroptiometry: a new method for the measurement of bone density. Brit J Radiol 62:587-592

Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J and Vogt TM (1993): Bone density at various sites for prediction of hip fractures. Lancet 341:72-75

Dahlöf CGH and Dimenäs E (1994): Migraine patients experience poorer subjective well-being/quality of life even between attacks. Cephalgia 15:31-36

Dean M, Carrington M and O'Brien SJ (2002): Balanced polymorphism selected by genetic versus infectious human disease. Annu Rev Genomics Hum Genet 3:263-292

Dicke WK (1950):Coeliakie. M.D. Thesis, Utrecht

Dickey W, Hughes DF and Mc Millan SA (2000): Reliance on serum endomysial antibody testing underestimates the true prevalence of coeliac disease by one fifth. Scand J Gastroenterol 35:181-183

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

Dieterich W, Laag E, Schöpper H, Volta U, Ferguson A, Gillett H, Riwcken E and Schuppan D (1998): Autoantibodies to tissue transglutaminase as predictors of celiac disease. Gastroenterol 115:1317-1321

Dimenäs E, Glise H, Hallenbäck B, Hernqvist H, Svedlund J and Wiklund I (1995): Well-being and gastrointestinal symptoms among patients referred to endoscopy owing to suspected duodenal ulcer. Scand J Gastroenterol 30:1046-1052

Dimenäs E, Carlsson H, Glise H, Israelsson B and Wiklund I (1996): Relevance of norm values as part of the documentation of quality of life instruments for use in upper gastrointestinal disease. Scand J Gastroenterol 31 Suppl:8-13

Doherty DG, Vaughan RW, Donaldson PT and Mowat AP (1992): HLA DQA, DQB, and DRB genotyping by oligonucleotide analysis: distribution of alleles and haplotypes in British caucasoids. Hum Immunol 34:53-63

Dupuy HJ (1984): Psychological general well-being (PGWB) index. In: Assessment of quality of life in clinical trials of cardiovascular therapies, pp. 170-183. Eds. Wenger NK, Matton ME, Furberg CF and Elinson J. Le Jacq Publishing Inc., New York

Ellis HJ, Rosen-Bronson S, O'Reilly N, and Ciclitira P (1998): Measurement of gluten using a monoclonal antibody to a coeliac toxic peptide of A-gliadin. Gut 43:190-195

Fabiani E and Catassi C (2001): The serum IgA class anti-tissue transglutaminase antibodies in the diagnosis and follow up of coeliac disease. Results of an international multi-centre study. International Working Group on Eu-tTG. Eur J Gastroenterol Hepatol 13:659-665

Fabiani E, Catassi C, Villari A, Gismondi P, Pierdomenico R, Ratsch IM, Coppa GV and Giorgi PL (1996): Dietary compliance in screening-detected coeliac disease adolescents. Acta Paediatr Suppl 412:65-67

Fabiani E, Taccari LM, Ratsch IM, Di Giuseppe S, Coppa GV and Catassi C (2000): Compliance with gluten-free diet in adolescents with screening-detected celiac disease: a 5-year follow-up study. J Pediatr 136:841-843

Farre C, Esteve M, Curcoy A, Cabre E, Arranz E, Amat LLand Garcia-Tornel S (2002): Hypertransaminasemia in pediatric celiac disease patients and its prevalence as a diagnostic clue. Am J Gastroenterol 97:3176-3181

Farthing MJ and Dawson AM (1983): Impaired semen quality in Crohn's disease--drugs, ill health, or undernutrition? Scand J Gastroenterol 18:57-60

Farthing MJ, Edwards CR, Rees LH and Dawson AM (1982): Male gonadal function in coeliac disease:1: Sexual dysfunction, infertility and semen quality. Gut 23:608-614

Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A and Goldblum SE (2000): Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet 355:1518-1519

Fasano A, Berti I, Gererduzzi T, Colletti RB, Drago S, Elitsur Y, Freen PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wassermann SS, Murray JA and Horvarth K (2003): Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 10:286-292

Ferguson A, Mc Clure JP and Townley RR (1976): Intraepithelial lymphocyte counts in small intestinal biopsies from children with diarrhoea. Acta Paediatr Scand 65:541-546

Ferguson A, Holmes GKT and Cooke WT (1982): Coeliac disease, fertility and pregnancy. Scand J Gastroenterol 17:65-68

Feldman M and Sears ER (1981): The wild gene resources of wheat. Scientific American 1:98-109

Fernandez A-M, Clerici N, Polanco I, Escobar H, Figueredo MA and de la Concha EG (1995): HLA-DQ alleles and susceptibility to celiac disease in Spanish children. Tissue Antigens 45:145-147

Ferreira M, Davies SL, Butler M, Scott D, Clark M and Kumar P (1992): Endomysial antibody: is it the best screening test for coeliac disease? Gut 33:1633-1637

Fickling WE, Mc Farlane XA, Bhalla AK and Robertson DA (2001): The clinical impact of metabolic bone disease in coeliac disease. Postgrad Med 77:33-36

Fitzpatrick KP, Sherman PM, Ipp M, Saunders N and Macarthur C (2001): Screening for celiac disease in children with recurrent abdominal pain. J Pediatr Gastroenterol Nutr 33:250-252

Floreani A, Betterle C, Baragiotta A, Martini S, Venturi C, Basso D, Pittoni M, Chiarelli S and Sategna-Guidetti C (2002): Prevalence of coeliac disease in primary biliary cirrhosis and of antimitochondrial antibodies in adult coeliac disease patients in Italy. Dig Liver Dis 34:248-250

Francis J, Carty JE and Scott BB (2002): The prevalence of coeliac disease in rheumatoid arthritis. Eur J Gastroenterol Hepatol 14:1355-1356

Frustaci A, Cuoco L, Chimenti C, Pieroni M, Fioravanti G, Gentiloni N, Maseri A and Gasbarrini G (2002): Celiac disease associated with autoimmune myocarditis. Circulation 105:2611-2618

Gee S (1888): On the coeliac disease. St Bart Hosp Rep 24:17-20

Giannotti A, Tiberio G, Castro M, Virgilii F, Colistro F, Ferretti F, Digilio MC, Gambarara M and Dallapiccola B (2001): Coeliac disease in Williams syndrome. J Med Genet 38:767-768

Glia A and Lindberg G (1997): Quality of life in patients with different typers of functional constipation. Scand J Gastroenterol 94:1782-1789

Gobbi G, Bouquet F, Greco L, Lambertini A, Tassari A, Ventura A and Zaniboni M (1990): Coeliac disease, epilepsy and cerebral calcifications. Lancet 340:439-443

Gomez JC, Selvaggio GS, Viola M, Pizarro B, la Motta G, de Barrio S, Castelleto R, Echeverria R, Sugai E, Vazquez H, Maurino E and Bai JC (2001): Prevalence of celiac disease in Argentina: screening of an adult population in the La Plata area. Am J Gastroenterol 96:2700-2704

Greco L, Corazza G, Babron MC, Clot F, Fulchignoni L, M.C., Percopo S, Zavattari P, Bouguerra F, Dib C, Tosi R, Troncone R, Ventura A, Mantavoni W, Magazzu G, Gatti R, Lazzaei R, Giunta A, Perri F, Iacono G, Cardi E, de Virgiliis S, Cataldo F, De Angelis G, Musumeci S and Clerget-Darpoux F (1998a): Genome search in celiac disease. Am J Hum Genet 62:669-675

Greco L, Percopo S, Clot F, Bouguerra F, Babron M-C, Eliaou J-F, Franzese C, Troncone R and Clerget-Darpoux F (1998b): Lack of correlation between genotype and phenotype in celiac disease. J Ped Gastr and Nutr 26:286-290

Greco L, Romino R, Coto I, Di Cosmo N, Percopo S, Maglio M, Paparo F, Gasperi V, Limongelli MG, Cotichini R, D'Agate C, Tinto N, Sacchetti L, Tosi R and Stazi MA (2002): The first large population based twin study of coeliac disease. Gut 50:624-628

Green JR, Goble HI, Edwards CR and Dawson AM (1977): Reversible insensitivity to androgens in men with untreated gluten enteropathy. Lancet 1:280-282

Greenberg CS, Birckbichler PJ and Rice RH (1991): Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. FASEB J 5:3071-3077

Grodzinsky E, Hed J, Lieden G, Sjögren F and Ström M (1990): Presence of IgA and IgG antigliadin antibodies in healthy adults as measured by micro-ELISA. Effect of various cutoff levels on specificity and sensitivity when diagnosing coeliac disease. Int Arch Allergy Appl Immunol 92:119-123

Hadjivassiliou MJ, Chattopadhyay AK, Davies-Jones GAB, Gibson A, Grunevald RA and Lobo AJ (1997): Neuromuscular disorder as a presenting feature of coeliac disease. J Neurol Neurosurg Psychiatry 63:770-775

Hadjivassilliou M, Grunewald R, Chattopadhaya AK, Davies-Jones GA, Gibson A, Jarratt JA, Kandler RH, Lobo A, Powell T and Smith CM (1998): Clinical, radiological, neurophysiological, and neuropathological characteristics of gluten ataxia. Lancet 352:1582-1585

Hallert C, Granno C, Grant C, Svensson H, Valdimarsson T and Wickström T (1998): Quality of life of adult coeliac patients treated for 10 years. Scand J Gastroenterol 33:933-938

Hallert C, Granno C, Hulten S, Midhagen G, Ström M, Swensson H and Valdimarsson T (2002): Living with coeliac disease: controlled study on the burden of illness. Scand J Gastroenterol 37:39-42

Hällström O (1989): Comparison of IgA-class reticulin and endomysium antibodies in coeliac disease and dermatitis herpetiformis. Gut 30:1225-1232

Halstensen TS, Scott H and Brandtzaeg P (1989): Intraepithelial T cells of the TcR gamma/delta+ CD8- and V delta 1/J delta 1+ phenotypes are increased in coeliac disease. Scand J Immunol 30:665-672

Halttunen T and Mäki M (1999): Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. Gastroenterology 116:566-572

Hansson T, Dahlbom I, Hall J, Holtz A, Elfman L, Dannaeus A and Klareskog L (2000): Antibody reactivity against human and guinea pig tissue transglutaminase in children with celiac disease. J Pediatr Gastroenterol Nutr 30:379-384

Hansson T, Dahlbom I, Rogberg S, Dannaeus A, Höpfl P, Gut H, Kraaz W and Klareskog L (2002): Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease. Ped Res 51:700-5

Hardman CM, Garioch JJ, Leonard JN, Thomas HJW, Walker MM, Lortan JE, Lister A and Fry L (1997): Absence of toxicity of oats in patients with dermatitis herpetiformis. New England J Med 337:1884-1887

Havelund T, Lind T, Wiklund I, Glise H, Hernqvist H, Lauritsen K, Lundell L, Pedersen SA, Carlsson R, Junghard O, Stubberod A and Anker-Hansen O (1999): Quality of life in patients with heartburn but without esophagitis: effects of treatment with omeprazole. Am J Gastroenterol 94:1782-1784

Henker J and Tandler C (1993): Epidemiologic studies of celiac disease in childhood in the Dresden district. Z Gastroenterol 31:716-718

Hervonen K, Hakanen M, Kaukinen K, Collin P and Reunala T (2002): First-degree relatives are frequently affected in coeliac disease and dermatitis herpetiformis. Scand J Gastroenterol 37:51-55

Hill I, Fasano A, Schwartz R, Counts M and Horvath K (2000): The prevalence of celiac disease in at-risk groups of children in the United States. J Pediatr 136:86-90

Hin H, Bird G, Fisher P, Mahy N and Jewell D (1999): Coeliac disease in primary care: case finding study. BMJ 18(7177):164-167

Holm K (1993a): Latent and silent coeliac disease. Acta Univ Tamperensis 371 (serA):1-90

Holm KH (1993b): Correlation of HLA-DR alleles to jejunal mucosal morphology in healthy first-degree relatives of coeliac disease patients. Eur J Gastroenterol Hepatol 5:35-39

Holmes GK (2002): Screening for coeliac disease in type 1 diabetes. Arch Dis Child 87:495-498

Holmes GKT, Prior P, Lane MR, Pope D and Allan RN (1989): Malignancy in coeliac disease - effect of a gluten free diet. Gut 30:333-338

Holopainen P, Mustalahti K, Uimari P, Collin P, Mäki M and Partanen J (2001): Candidate gene regions and genetic heterogeneity in gluten sensitivity. Gut 48:696-701

Howard MR, Turnbull AJ, Morley P, Hollier P, Webb R and Clarke A (2002): A prospective study of the prevalence of undiagnosed coeliac disease in laboratory defined iron and folate deficiency. J Clin Pathol 55:754-757

Hovdenak N, Hovlid E, Aksnes L, Fluge G, Erichsen MM and Eide J (1999): High prevalence of asymptomatic coeliac disease in Norway:a study of blood donors. Eur J Gastroenterol Hepatol 11:185-187

Hovell CJ, Collett JA, Vautier G, Cheng AJ, Sutanto E, Mallon DF, Olynyk JK and Cullen DJ (2001): High prevalence of coeliac disease in a population-based study from Western Australia: a case for screening. Med J Aust 175:247-250

Howell WM, Leung ST, Jones DB, Nakshabendi I, Hall MA, Lanchburry JS, Ciclitira PJ and Wright DH (1995): HLA-DRB, -DQA, and DQB polymorphism in celiac disease and enteropathy associated T-cell lymphoma. Common features and additional risk factors for malignancy. Hum Immunol 43:29-37

Iltanen S, Holm K, Eland C, Partanen J and Mäki M (1997): Markers of coeliac disease latency in children having normal jejunal morphology. J Pediatr Gastroenterol Nutr 24:453

Iltanen S, Holm K, Ashorn M, Ruuska T, Laippala PM and Maki M (1999a): Changing jejunal gamma delta T cell receptor (TCR)-bearing intraepithelial lymphocyte density in coeliac disease. Clin Exp Immunol 117(1):51-55

Iltanen S, Holm K, Partanen J, Laippala P and Mäki M (1999b): Increased density of jejunal gammadelta+ T cells in patients having normal mucosa--marker of operative autoimmune mechanisms? Autoimmunity 29(3):179-187

Imanishi T (1992): Allele and haplotype frequencies for HLA and complement loci in carious ethnic groups. In Tsuji, K. Aizawa, M. and Sasazuki, T. eds. HLA 1991. Oxford University Press, Oxford, UK. pp. 1065-1220

Ivarsson A, Persson LA, Juto P, Peltonen M, Suhr O and Hernell O (1999a): High prevalence of undiagnosed coeliac disease in adults: a Swedish population based study. J Intern Med 245:63-68

Ivarsson SA, Carlsson A, Bradberg A, Alm J, Aronsson S, Gustafsson J, Hagenäs L, Hager A, Kristrom B, Marcus C, Moell C, Nilsson KO, Tuvemo T, Westphal O, Albetsson-Wikland K and Aman J (1999b): Prevalence of coeliac disease in Turner syndrome. Acta Paediatr 88:933-936

Ivarsson A, Hernell O, Stenlund H and Persson LA (2002): Breast-feeding protects against celiac disease. Am J Clin Nutr 75:914-921

Jachuck SJ, Brierly H, Jachuck S and Willcox PM (1982): The effect of hypotensive drugs on the quality of life. J R coll Gen Pract 32:103-105

Janatuinen EK, Pikkarainen PH, Kemppainen TA, Kosma VM, Järvinen RM, Uusitupa MI and Julkunen RJ (1995): A comparison of diets with and without oats in adults with celiac disease. New Engl J Med 333:1033-1037

Janatuinen EK, Kemppainen TA, Julkunen RJ, Kosma VM, Mäki M, Heikkinen M and Uusitupa MI (2002): No harm from five year ingestion of oats in coeliac disease. Gut 50:332-335

Johnston SD, Watson RG, Mc Millan SA, Sloan J and Love AH (1998): Coeliac disease detected by screening is not silent - simply unrecognized. QJM 91:853-860

Joint FAO/WHO Food Standards Programme (1981): Codex Alimentarius Commission Codex Stan:118

Kagnoff MF, Austin RK, Hubert JJ, Bernardin JE and Kasarda DD (1984): Possible role for a human adenovirus in the pathogenesis of celiac disease. J Exp Med 160:1544-1557

Kalayci AG, Kansu A, Girgin N, Kucuk O and Aras G (2001): Bone mineral density and importance of a gluten-free diet in patients with celiac disease in childhood. Pediatrics 108:E89

Karell K, Klinger N, Holopainen P, Levo A and Partanen J (2000): Major histocompatibility complex (MHC)-linked microsatellite markers in a founder population. Tissue Antigens 56:45-51

Kaukinen K, Collin P, Holm K, Rantala I, Vuolteenaho N, Reunala T and Mäki M (1999): Wheat starch-containing gluten-free flour products in the treatment of coeliac disease and dermatitis herpetiformis. A long-term follow-up study. Scand J Gastroenterol 34:163-169

Kaukinen K, Turjanmaa K, Mäki M, Partanen J, Venäläinen R, Reunala T and Collin P (2000): Intolerance to cereals is not specific for celiac disease. Scand J Gastro 35:942-946

Kaukinen K, Mäki M, Partanen J, Sievänen H and Collin P (2001): Celiac disease without villous atrophy: revision of criteria called for. Dig Dis Sci 46:879-887

Kaukinen K, Halme L, Collin P, Färkkilä M, Mäki M, Vehmanen P, Partanen J and Hockerstedt K (2002a): Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. Gastroenterology 123:2158-2159

Kaukinen K, Partanen J, Mäki M and Collin P (2002b): HLA-DQ typing in the diagnosis of celiac disease. Am J Gastroenterol 97:695-699

Kemppainen T, Kroger H, Janatuinen E, Arnala I, Kosma VM, Pikkarainen P, Julkunen R, Jurvelin J, Alhava E and Uusitupa M (1999): Osteoporosis in adult patients with celiac disease. Bone 24:249-255

Kero J, Gissler M, Hemminki E and Isolauri E (2001): Could TH1 and TH2 diseases coexist? Evaluation of asthma incidence in children with coeliac disease, type 1 diabetes, or rheumatoid arthritis: a register study. J Allergy Clin Immunol 108:781-783

Keusch GT (2003): The history of nutrition: malnutrition, infection and immunity. J Nutr 133:336-340S

Kilander AF, Dotevall G, Fallström SP, Gillberg RE, Nilsson LA and Tarkowski A (1983): Evaluation of gliadin antibodies for detection of coeliac disease. Scand J Gastroenterol 18:377-383

King AL, Yiannakou JY, Brett PM, Curtis D, Morris MA, Dearlove AM, Rhodes M, Rosen-Bronson S, C. M, Ellis HJ and Ciclitira PJ (2000): A genome-wide family-based linkage study of coeliac disease. Ann Hum Genet 64:479-490

Kluge H, Koch HK, Grosse-Wilde H, Lesch R and Gerok W (1982): Follow-up of treated adult celiac disease; clinical and morphological studies. Hepato-Gastroenterol 29:17-23

Koistinen J (1975): Selective IgA-deficiency in blood donors. Vox sang 29:192-202

Kolho KL, Färkkilä M and Savilahti E (1998): Undiagnosed coeliac disease is common in Finnish adults. Scand J Gastroenterol 33:1280-1283

Kolho KL, Tiitinen A, Tulppala M, Unkila-Kallio L and Savilahti E (1999): Screening for coeliac disease in women with a history of miscarriage or infertility. Br J Obstet Gynaecol 106:171-173

Kolsteren MM, Koopman HM, Schalekamp G and Mearin ML (2001): Health-related quality of life in children with celiac disease. J Pediatr 138:593-595

Korponay-Szabo I, Kovacs J, Lorincz M, Torok E and Goracz G (1998): Families with multiple cases of gluten-sensitive enteropathy. Z Gastroenterol 36:553-558

Korponay-Szabo IR, Kovacs J, Czinner A, Goracz G, Vamos A and Szabo T (1999): High prevalence of silent coeliac disease in preschool children screened with IgA/IgG anti-endomysium antibodies. J Pediatric Gastroent Nutr 28:26-30

Korponay-Szabo I, Sulkanen S, Halttunen T, Maurano F, Rossi M, Mazzarella G, Laurila K, Troncone R and Mäki M (2000): Tissue transglutaminase is the target in both rodent and primate tissues for celiac disease-specific autoantibodies. J Pediatr Gastroenterol Nutr 31:520-527

Kuitunen P, Kosnai I and Savilahti E (1982): Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. J Pediatr Gastroenterol Nutr 1:525-531

Kumar PJ, Walker-Smith J, Milla P, Harris G, Colyer J and Halliday R (1988): The teenage coeliac: follow up study of 102 patients. Arch Dis Child 63:916-920

Kutlu T, Brousse N, Rambaud C, Le Deist F, Schmitz J and Cerf-Bensussan N (1993): Numbers of T cell receptor (TCR) alpha beta+ but not of TcR gamma delta+ intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet. Gut 34:208-214

Ladinser B, Rossipal E and Pittschieler K (1994): Endomysium antibodies in coeliac disease: an improved method. Gut 35:776-778

Lagerqvist C, Ivarsson A, Juto P, Persson LA and Hernell O (2001): Screening for adult coeliac disease - which serological marker(s) to use? J Intern Med 250:241-248

Lähdeaho ML, Lehtinen M, Rissa HR, Hyöty H, Reunala T and Mäki M (1993): Antipeptide antibodies to adenovirus E1b protein indicate enhanced risk of celiac disease and dermatitis herpetiformis. In Arch Allergy Immunol 101:272-276

Lampis R, Morelli L, Congia M, Macis MD, Mulargia A, Loddo M, De Virgiliis S, Marrosu MG, Todd JA and Cucca F (2000): The inter-regional distribution of HLA class II haplotypes indicates the suitability of the Sardinian population for case-control association studies in complex diseases. Hum Mol Genet 9:2959-2965

Larizza D, Calcaterra V, De Giacomo C, De Silvestri A, Asti M, Badulli C, Autelli M, Coslovich E and Martinetti M (2001): Celiac disease in children with autoimmune thyroid disease. J Pediatr 139:738-740

Leon F, Camarero C, R-Pena R, Eiras P, Sanchez L, Baragano M, Lombardia M, Bootello A and Roy G (2001): Anti-transglutaminase IgA ELISA: clinical potential and drawbacks in celiac disease diagnosis. Scan J Gastroenterol 36:849-853

Lerner A, Kumar V and Iancu TC (1994): Immunological diagnosis of childhood coeliac disease: comparison between antigliadin, antireticulin and antiendomysial antibodies. Clin Exp Immunol 95:78-82

Lindh E, Ljunghall S, Larsson K and Lavo B (1992): Screening for antibodies against gliadin in patients with osteoporosis. J Intern Med 231:403-406

Liu J, Juo SH, Holopainen O, Terwilliger J, Tong X, Grunn A, Brito M, Green P, Mustalahti K, Mäki M, Gilliam TC and Partanen J (2002): Genomewide linkage analysis of celiac disease in Finnish families. Am J Hum Genet 70:51-59

Lock RJ, Pitcher MC and Unsworth DJ (1999): IgA anti-tissue transglutaminase as a diagnostic marker of gluten sensitive enteropathy. J Clin Pathol 52:274-277

Luostarinen L, Pirttilä T and Collin P (1999): Coeliac disease presenting with neurological disorders. Eur Neurol 42:132-135

Luostarinen L, Dastidar P, Collin P, Peräaho M, Mäki M, Erilä T and Pirttilä T (2001): Association between coeliac disease, epilepsy and brain atrophy. Eur Neurol 46:187-191

Lydick E and Epstein RS (1993): Interpretation of quality of life changes. Qual Life Res 2:221-226

MacGowan DJ, Hourihane DO, Tanner WA and O'Morain C (1996): Duodeno-jejunal adenocarcinoma as a first presentation of coeliac disease. J-Clin-Pathol 49:602-604

Mäki M, Hällström O, Vesikari T and Visakorpi JK (1984): Evaluation of a serum IgA-class reticulin antibody test for the detection of childhood celiac disease. J Pediatr 105:901-905

Mäki M, Holm K, Koskimies S, Hällström O and Visakorpi JK (1990): Normal small bowel biopsy followed by coeliac disease. Arch Dis Child 65:1137-1141

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

Mäki M and Collin P (1997): Coeliac disease. Lancet 349:1755-1759

Marsh MN (1992): Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology 102:330-354

Marsh MN (1993): Gluten sensitivity and latency: can patterns of intestinal antibody secretion define the great 'silent majority?' Gastroenterology 104:1550-1554

Martinelli P, Troncone R, Paparo F, Torre P, Trapanese E, Fasano C, Lamberti A, Budillon G, Nardone G and Greco L (2000): Coeliac disease and unfavourable outcome of pregnancy. Gut 46:332-335

Mayer M, Greco L, Troncone R, Auricchio S and Marsh MN (1991): Compliance of adolescents with coeliac disease with a gluten free diet. Gut 32:881-885

Mazure R, Vazquez H, Gonzalez D, Mautalen C, Pedreira S, Boerr L and Bai JC (1994): Bone mineral affection in asymptomatic adult patients with celiac disease. Am J Gastroenterol 89:2130-2134

Mazzilli MC, Ferrante P, Mariani E, Petronzelli F, Triglione P and Bonamico M (1992): A study of Italian pediatric celiac disease confirms that the primary HLA association is to the DQ(alpha 1*0501, beta 1*0201) heterodimer. Hum Immunol 33:133-139

Mearin ML, Biemond I, Pena AS, Polanco I, Vazquez C, Schreuder GT, de Vries RR and van Rood JJ (1983): HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease. Gut 24:532-537

Meloni G, Dore A, Fanciulli G, Tanda F and Bottazzo G (1999a): Subclinical coeliac disease in schoolchildren from northern Sardinia. Lancet 353(9146):37

Meloni GF, Dessole S, Vargiu N, Tomasi PA and Musumeci S (1999b): The prevalence of coeliac disease in infertility. Hum Reprod 14:2759-2761

Meyer D, Stavropoulos S, Diamond B, Shane E and Green PH (2001): Osteoporosis in a North American adult population with celiac disease. Em J Gastroenterol 96:112-119

Molberg O, Mc Adam SN and Sollid LM (2000): Role of tissue transglutaminase in celiac disease. J Pediatr Gastroenterol Nutr 30:232-240

Molteni N, Bardella MT and Bianchi PA (1990): Obstetric and gynecological problems in women with untreated celiac sprue. J Clin Gastroenterol 12:37-39

Montgomery AM, Goka AK, Kumar PJ, Farthing MJ, and Clark ML (1988): Low gluten diet in the treatment of adult coeliac disease: effect on jejunal morphology and serum anti-gluten antibodies. Gut 29:1564-1568

Mora S, Barera G, Ricotti A, Weber G, Bianchi C and Chiumello G (1998): Reversal of low bone density with a gluten-free diet in children and adolescents with celiac disease. Am J Clin Nutr 67:477-481

Naluai AT, Nilsson S, Gudjonsdottir AH, Louka AS, Ascher H, Ek J, Hallberg B, Samuelsson L, Kristiansson B, Martinsson T, Nerman O, Sollid LM and Wahlström J (2001): Genome-wide linkage analysis of Scandinavian affected sib-pairs supports presence of susceptibility loci for celiac disease on chromosomes 5 and 11. Eur J Hum Genet 9:938-944

Naughton MJ and Wiklund I (1993): A critical review of dimension specific measures of health-related quality of life in cross-cultural research. Q Life Res 2:397-432

Nejentsev S, Sjöroos M, Soukka T, Knip M, Simll O, Lövgren T and Ilonen J (1999): Population-base genetic screening for the estimation of Type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. Diabet Med 16:985-992

Not T, Ventura A, Peticarari S, Basile S, Torre G and Dragovic D (1993): A new, rapid, noninvasive screening test for coeliac disease. J Ped 123:425-427

Not T, Horvath K, Hill I, Partanen J, Hammed A, Magazzu G and Fasano A (1998): Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. Scand J Gastroenterol 33(5):494-498

Nuti R, Martini G, Valenti R, Giovani S, Salvadori S and Avanzati A (2001): Prevalence of undiagnosed coeliac syndrome in osteoporotic women. J Intern Med 250:361-366

O'Farrelly C, Kelly J, Hekkens W, Bradley B, Thompson A, Feighery C and Weir DG (1983): Alpha gliadin antibody levels: a serological test for coeliac disease. BMJ 286:2007-2010

Petaros P, Martelossi S, Tommasini A, Torre G, Caradonna M and Ventura A (2002) Prevalence of autoimmune disorders in relatives of patients with celiac disease. Dig Dis Sci 47:1427-1431

Ploski R, Thorsby E and Sollid LM (1993): On the HLA-DQ (alpha 1*0501, beta 1*0201)- assiciated susceptibility in celiac disease: a possible gene dosage affect of DQB1*0201. Tissue Antigens 41:173-177

Pococco M and Ventura A (1995): Coeliac disease and insulin-dependent diabetes mellitus: a causal association? [see comments]. Acta Paediatr 84:1432-1433

Polanco I, Biemond I, Van Leeuwen A, Schreuder I, Kahn PM, Guerrero J, D'Amaro J, Vasquez C, van Rood JJ and Pena AS (1981): Gluten sensitive enteropathy in Spain: genetic and environmental factors. In: McConnell RB, ed. The genetics of coeliac disease. Lancaster, England: MTB.:211-231

Polvi A, Eland C, Koskimies S, Mäki M and Partanen J (1996): HLA DQ and DP in Finnish families with coeliac disease. Eur J Immunogen 23:221-234

Polvi A, Arranz E, Fernandez-Arquero M, Collin P, Maki M, Sanz A, Calvo C, Maluenda C, Westman P, de la Concha EG and Partanen J (1998): HLA-DQ2-negative celiac disease in Finland and Spain. Hum Immunol 59:169-75

Popat S, Bevan S, Braegger CP, Busch A, O'Donoghue D, Falth-Magnusson K, Godkin A, Högberg L, Holmes G, Hosie KB, Howdle PD, Jenkins H, Jewell D, Johnston S, Kennedy NP, Kumar P, Logan RF, Love AH, March MN, Mulder CJ, Sjöberg K, Stenhammar L, Walker-Smith J and Houlston RS (2002): Genome screening of coeliac disease. J Med Genet. 39:328-331

Prati D, Bardella MT, Peracchi M, Porretti L, Cardillo M, Pagliari C, Tarantino C, Della TE, Scalamogna M, Bianchi PA, Sirchia G and Conte D (2002): High frequency of anti-endomysial reactivity in candidates to heart transplant. Dig Liver Dis 34:13-15

Prince HE, Norman GL and Binder WL (2000): Immunoglobulin A (IgA) deficiency and alternative celiac disease-associated antibodies in sera submitted to a reference laboratory for endomysial IgA testing. Clin Diagn Lab Immunol 7:192-196

Ransford RA, Hayes M, Palmer M and Hall MJ (2002): A controlled, prospective screening study of celiac disease presenting as iron deficiency anemia. J Clin Gastroenterol 35:228-233

Reunala T, Kosnai I, Karpati S, Kuitunen P, Török E and Savilahti E (1984): Dermatitis herpetiformis: jejunal findings and skin response to gluten-free diet. Arch Dis Child 59:517-522

Reunala T, Collin P, Holm K, Pikkarainen P, Miettinen A, Vuolteenaho N and Mäki M (1998): Tolerance to oats in dermatitis herpetiformis. Gut 43:490-493

Revicki DA, Wood M, Wiklund I and Crawley J (1998): Reliability and validity of the Gastrointestinal Symptom Rating Scale in patients with gastroesophageal reflux disease. Qual Life Res 7:75-83

Riestra S, Fernandez E, Rodrigo L, Garcia S and Ocio G (2000): Prevalence of coeliac disease in the general population of northern Spain. Strategies of serologic screening. Scand J Gastroenterol 35:398-402

Risch N (1987): Assessing the role of HLA-linked and unlinked determinants of disease. Am J Hum Genet 40:1-14

Rostami K, Mulder C, Werre J, van Beukelen F, Kerchhaert J, Crusius J, Pena A, Willekens F and Meijer J (1999): High prevalence of celiac disease in apparently healthy blood donors suggests a high prevalence of undiagnosed celiac disease in the Dutch population. Scand J Gastroenterol 34(3):276-279

Rostami K, Mulder CJ, van Overbeek FM, Kerckhaert J, Meijer JW, von Blomberg MB and Heymans HS (2000): Should relatives of coeliacs with mild clinical complaints undergo a small-bowel biopsy despite negative serology? Eur J Gastroenterol Hepatol 12:51-55

Rönningen KS, Spurkland A, Markussen G, Iwe T, Vartdal F and Thorsby E (1990): Distribution of HLA class II alleles among Norwegian Caucasians. Hum Immunol 29:275-281

Sacchetti L, Ferrajolo A, Salerno G, Esposito P, Lofrano MN, Oriani G, Micillo M, Paparo F, Troncone R, Auriccio S and Salvatore F (1996): Diagnostic value of various serum antibodies detected by diverse methods in childhood celiac disease. Clin Chem 42:1838-1842

Sategna-Guidetti C, Grosso S, Bruno M and Grosso SB (1995): Comparison of serum anti-gliadin, anti-endomysium, and anti-jejunum antibodies in adult celiac sprue. J Clin Gastroenterol 20:17-21

Sategna-Guidetti C, Grosso SB, Bruno M and Grosso S (1997): Is human umbilical cord the most suitable substrate for the detection of endomysium antibodies in the screening and follow-up of coeliac disease? Eur J Gastoenterol Hepatol 9(7):657-660

Sategna-Guidetti C, Grosso SB, Grosso S, Mengossi G, Aimo G, Zaccaria T, Di Stefano M and Isaia GC (2000): The effect of 1-year gluten withdrawal on bone mass, bone metabolism and nutritional status in newly diagnosed adult coeliac disease patients. Aliment Pharmacol Ther 14:35-43

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

Savilahti E, Pelkonen P and Visakorpi JK (1971): IgA deficiency in children. A clinical study with special reference to intestinal findings. Arch Dis Child 46:665-670

Savilahti E, Viander M, Perkkiö M, Vainio E, Kalimo K and Reunala T (1983): IgA antigliadin antibodies: a marker of mucosal damage in childhood coeliac disease. Lancet 1:320-322

Savilahti E, Pelkonen P, Verkasalo M and Koskimies S (1985) Selective deficiency of immunoglobulin A in children. Klin Padiatr 197:336-340

Savilahti E, Arato A and Verkasalo M (1990): Intestinal gamma/delta receptor-bearing T lymphocytes in celiac disease and inflammatory bowel diseases in children. Constant increase in celiac disease. Pediatr Res 28:579-581

Savilahti E, Örmälä T, Arato A, Hacek G, Holm K, Klemola T, Nemeth A, Mäki M and Reunala T (1997): Density of gamma/delta T cells in jejunal epithelium of patients with coeliac disease and dermatitis herpetiformis is increased with age. Clin Exp Immunol 109:464-467

Sblattero D, Berti I, Trevisiol C, Marzari R, Tommasini A, Bradbury A, Fasano A, Ventura A and Not T (2000): Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. Am J Gastroenterol 95:1253-1257

Schuppan D (2000): Current concepts of celiac disease pathogenesis. Gastroenterology 119:234-242

Scotta MS, Salvatore S, Salvatoni A, De Amici M, Ghiringhelli D, Broggini M and Nespoli L (1997): Bone mineralization and body composition in young patients with celiac disease. Am J Gastroenterol 92:1331-1334

Seah PP, Fry LL, Rossiter MA, Hoffbrand AV and Holborow E (1971): Anti-reticulin antibodies in childhood coeliac disease. Lancet I:834-836

Selby WS, Painter D, Collins A, Faulkner-Hogg KB and Loblay RH (1999): Persistent mucosal abnormalities in coeliac disease are not related to the ingestion of trace amounts of gluten. Scand J Gastroenterol 34:909-914

Shamir R, Lerner A, Shinar E, Lahat N, Sobel E, Bar-or R, Kerner H and Eliakin R (2002): The use of a single serological marker underestimates the prevalence of celiac disease in Israel: a study of blood donors.

Sher KS and Mayberry JF (1996): Female fertility, obstetric and gynaegological history in coeliac disese: a case control study. Acta Paediatr Suppl 412:76-77

Sievänen H, Kannus P, Nieminen V, Heinonen A, Oja P and Vuori I (1996): Estimation of various mechanical characteristics of human bone using dual energy X-ray absorptiometry: methodology and precision. Bone 18:17S-27S

Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F and Thorsby E (1989): Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. J Exp Med 169:345-350

Sollid LM and Thorsby E (1990): The primary association of celiac disease to a given HLA-DQ alpha/beta heterodimer explains the divergent HLA-DR associations observed in various Caucasian populations. Tissue Antigens 36:136-137

Spencer J, Isaacson PG, Diss TC and MacDonald TT (1989): Expression of disulfide-linked and non-disulfide-linked forms of the T cell receptor gamma/delta heterodimer in human intestinal intraepithelial lymphocytes. Eur J Immunol 19:1335-1338

Spencer J, Isaacson PG, MacDonald TT, Thomas AJ and Walker-Smith JA (1991): Gamma/delta T cells and the diagnosis of coeliac disease. Clin Exp Immunol 85:109-113

Spurkland A, Sollid LM, Polanco I, Vartdal F and Thorsby E (1992): HLA-DR and -DQ genotypes of celiac disease patients serologically typed to be non-DR3 or nonDR5/7. Hum Immunol 35:188-192

Stenhammar L, Fallström SP, Jansson G, Jansson U and Lindberg T (1986): Coeliac disease in children of short stature without gastrointestinal symptoms. Eur J Pediatr 145:185-186

Stern M (2000): Comparative evaluation of serologic tests for celiac disease: A European initiative toward standardization. J Pediatr Gastroenterol Nutr 31:513-519

Stern M, Fisher K and Gruttner R (1979): Immunofluorescent serum gliadin antibodies in children with coeliac disease and carious malabsorptive disorders. Eur J Pediatrics 130:155-164

Sulkanen S, Collin P, Laurila K and Mäki M (1998a): IgA- and IgG-class antihuman umbilical cord antibody tests in adult coeliac disease. Scand J Gastroenterol 33:251-254

Sulkanen S, Halttunen T, Laurila K, Kolho KL, Korponay- Szabo IR, Sarnesto A, E. S, Collin P and Mäki M (1998b): Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 115(6):1322-1328

Svedlund J, Sjödin I and Dotevall G (1988): GSRS - a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci 33:129-134

Söderström K, Bucht A, Halapi E, Gronberg A, Magnusson I and Kiessling R (1996): Increased frequency of abnormal gamma delta T cells in blood of patients with inflammatory bowel diseases. J Immunol 156:2331-2339

Tighe MR, Hall MA, Barbado M, Cardi E, Welsh KI and Ciclitira PJ (1992): HLA class II alleles associated with celiac disease susceptibility in a southern European population. Tissue Antigens 40:90-97

Tighe MR, Hall MA, Ashkenazi A, Siegler E, Landbury JS and Ciclitira PJ (1993): Coeliac disease among Ashkenazi Jews from Israel. A study of the HLA class II alleles and their associations with disease susceptibility-. Hum Immunol 34:47-52

Trejdosiewicz LK and Howdle PD (1995): T-cell responses and cellular immunity in coeliac disease. In: Bailliere's Clinical Gastroenterology, pp. 251-72. Eds. Howdle P.D., Arhur M.J.P., Bouchier I.A.D., Carter D.C., Creutzfeldt W., Dent J., Gracey M., Tytgat G.N.J. Bailliere Tindall, London.

Trejdosiewicz LK, Calabrese A, Smart CJ, Oakes DJ, Howdle PD, Crabtree JE, Losowsky MS, Lancaster F and Boylston AW (1991): Gamma delta T cell receptor-positive cells of the human gastrointestinal mucosa: occurrence and V region gene expression in Heliobacter pylori-associated gastritis, coeliac disease and inflammatory bowel disease. Clin Exp Immunol 84:440-444

Trevisiol C, Ventura A, Baldas V, Tomassini A, Santon D, Martelossi S, Torre G, Berti I, Spano A, Grovella S, Amoroso A, Sblattero D, Marzari R, Bradbury A and Not T (2002): A reliable screening procedure for coeliac disease in clinical practice. Scand J Gastroenterol 37:679-684

Troncone R (1995): Latent coeliac disease in Italy. Acta Paediatr 84:1252-1257

Troncone R and Ferguson A (1991): Anti-gliadin antibodies. J Pediatr Gastroenterol Nutr 12:150-158

Troncone R, Maurano F, Rossi M, Micillo M, Greco L, Auriccio R, Salerno G, Salvatore F and Sacchetti L (1999): IgA antibodies to tissue transglutaminase: An effective diagnostic test for celiac disease. J Pediatr 134(2):166-171

Tursi A, Giorgetti G, Brandimarte G, Rubino E, Lombardi D and Gasbarrini G (2001): Prevalence and clinical presentation of subclinical/silent celiac disease in adults: an analysis on a 12 -year observation. Hepatogastroenterology 48:462-464

Usai P, Minerba L, Marini B, Cossu R, Spada S, Carpiniello B, Cuomo R and Boy MF (2002): Case control study on health-related quality of life in adult coeliac disease. Dig Liver Dis 34:547-552

Wahab PJ, Meijer JW and Mulder CJ (2002): Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. Am J Clin Pathol 118:459-463

Valdimarsson T, Franzen L, Grodzinsky E, Skogh T and Ström M (1996a): Is small bowel biopsy necessary in adults with suspected celiac disease and IgA anti-endomysium antibodies? 100% positive predictive value in adults. Dig Dis Sci 41:83-87

Valdimarsson T, Löfman O, Toss G and Ström M (1996b): Reversal of osteopenia with diet in adult coeliac disease. Gut 38:322-327

Valentino R, Savastano S, Tommaselli AP, Dorato M, Scarpitta MT, Gigante M, Micillo M, Paparo F, Petrone E, Lombardi G and Troncone R (1999): Prevalence of coeliac disease in patients with thyroid autoimmunity. Horm Res 51:124-127

Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH and Visakorpi JK (1990): Revised criteria for diagnosis of coeliac disease. Arch Dis Child 65:909-911

Walters JR (1994): Bone mineral density in coeliac disease. Gut 35:150-151

Vasquez H, Mazure R, Gonzales D, Flores D, Pedreire S, Niveloni S, Smecuol E, Maurino E and Bai JC (2000): Risk of fractures in celiac disease patients: a cross-sectional, case-control study. Am J Gastroenterol 95:183-189

Weijers HA, Lindquist B, Anderson CM, Rey J, Shmerling DH, Visakorpi JK, Hadorn B and Gruttner R (1970): Diagnostic criteria in coeliac disease. Acta Paediatr Scand 59:461-463

Weile B, Cavell B, Nivenius K and Krasilnikoff PA (1995): Striking differences in the incidence of childhood celiac disease between Denmark and Sweden: a plausible explanation. J Pediatr Gastroenterol Nutr 21:64-68

Weile B, Heegaard NH, Hoier-Madsen M, Wiik A and Krasilnikoff PA (2002): Tissue transglutaminase and endomysial autoantibodies measured in an historical cohort of children and young adults in whom coeliac disease was suspected. Eur J Gastroenterol Hepatol 14:71-76

Weinstein RS and Manolagas SC (2000): Apoptosis and osteoporosis. Am J Med 108:153-164

Weinstein WM (1974): Latent celiac sprue. Gastroenterology 66:489-493

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

West J, Lloyd CA, Hill PG and Holmes GK (2002): IgA-antitissue transglutaminase: validation of a commercial assay for diagnosing coeliac disease. Clin Lab 48:241-246

Wiklund I (1990): Measuring quality of life in medicine. Scand J Prim Healgh Care Suppl 1:11-14

Vitoria JC, Arrieta A, Arranz C, Ayesta A, Sojo A, Maruri N and Garcia-Masdevall MD (1999): Antibodies to gliadin, endomysium, and tissue transglutaminase for the diagnosis of celiac disease. J Ped Gastr Nutr 29:571-574

Vogelsang H, Genser D, Wyatt J, Lochs H, Ferenci P, Granditsch G and Penner E (1995): Screening for celiac disease: a prospective study on the value of noninvasive tests. Am J Gastroenterol 90:394-398

Volta U, Molinaro N, Fusconi M, Cassani F and Bianchi FB (1991): IgA antiendomysial antibody test. A step forward in celiac disease screening. Dig Dis Sci 36:752-756

Volta U, De Franceschi L, Lari F, Molinaro N, Zoli M and Bianchi FB (1998a): Coeliac disease hidden by cryptogenic hypertransaminasemia. Lancet 352:26-29

Volta U, De Franceschi L, Molinaro N, Cassani F, Muratori L, Lenzi M, Bianchi FB and Czaja AJ (1998b): Frequency and significance of anti-gliadin and anti-endomysial antibodies in autoimmune hepatitis. Dig Dis Sci 43(10):2190-2195

Volta U, Bellentani S, Bianchi FB, De Franceschi L, Miglioli L, Granito A, Balli F and Tiribelli C (2001a) High prevalence of celiac disease in Italian general population. Dig dis sci 46:1500-1505

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

Volta U, Rodrigo L, Granito A, Petrolini N, Muratori P, Linares A, Veronesi L, Fuentes D, Zauli D and Bianchi FB (2002): Coeliac disease in autoimmune cholestatic liver disorders. Am J Gastroenterol 97:2609-2613

Wolters V, Vooijs-Moulaert AF, Burger H, Brooimans R, De Shryver J, Rijkers G and Houwen R (2002): Human tissue transglutaminase enzyme linked immunosorbent assay outperforms both the guinea-pig based tissue transglutaminase assay and anti-endomysium antibodies when screening for coeliac disease. Eur J Ped 161:284-287

Wong RC, Wilson RJ, Steele RJ, Radford-Smith G and Adelstein S (2002): A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits. J Clin Pathol 55:488-494

Woolley N, Holopainen P, Ollikainen V, Mustalahti K, Mäki M, Kere J and Partanen J (2002): A new locus for coeliac disease mapped to chromosome 15 in a population isolate. Hum Genet 111:40-45

Zhong F, McCombs CC, Olson JM, Elston RC, Stevens FO, McCarthy CF, Michalski JP (1996) An autosomal screen for genes that predispose to celiac disease in the western counties of Ireland. Nature Genet 14:329-333

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