The prevalence of celiac disease in Europe Results of a centralized international mass screening project

Mustalahti, K., Catassi, C., Reunanen, A., Fabiani, E., Heier, M., McMillan, S., ... Watson, P. (2010). The prevalence of celiac disease in Europe Results of a centralized international mass screening project. Annals of Medicine, 42(8), 587-595. DOI: 10.3109/07853890.2010.505931

Published in:
Annals of Medicine

Annals of Medicine, 2010; 42: 587–595

informa
healthcare

ORIGINAL ARTICLE

The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project

KIRSI MUSTALAHTI[1], CARLO CATASSI[2,3], ANTTI REUNANEN[4], ELISABETTA FABIANI[2], MARGIT HEIER[5], STAN MCMILLAN[6], LIAM MURRAY[7], MARIE-HELENE METZGER[5], MAURIZIO GASPARIN[8], ENZO BRAVI[8], MARKKU MÄKI[1] AND THE MEMBERS OF THE COELIAC EU CLUSTER, EPIDEMIOLOGY

[1]*Paediatric Research Centre, University of Tampere and Department of Paediatrics, Tampere University Hospital, Tampere, Finland,* [2]*Department of Pediatrics, Università Politecnica delle Marche, Ancona, Italy,* [3]*Center For Celiac Research, University of Maryland, School of Medicine, Baltimore, MD, USA,* [4]*National Public Health Institute, Helsinki, Finland,* [5]*Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Germany,* [6]*Regional Immunology Laboratory, Royal Hospitals, Belfast, UK,* [7]*Department of Epidemiology and Public Health, Queen's University, Belfast, UK, and* [8]*Eurospital SpA, Trieste, Italy*

Abstract

Introduction. Although the prevalence of celiac disease (CD) has been extensively investigated in recent years, an accurate estimate of CD frequency in the European population is still lacking. The aims of this study were: 1) to establish accurately the prevalence of CD in a large sample of the European population (Finland, Germany, Italy, and UK), including both children and adults; and 2) to investigate whether the prevalence of CD significantly varies between different areas of the European continent.

Materials and methods. Samples were drawn from the four populations. All 29,212 participants were tested for CD by tissue transglutaminase (tTG) antibody test. Positive and border-line findings were further tested for serum endomysial antibodies (EMA). All serological determinations were centrally performed. Small-bowel biopsies were recommended to autoantibody-positive individuals. Previously diagnosed cases were identified.

Results. The overall CD prevalence (previously diagnosed plus anti-tTG and EMA positives) was 1.0% (95% CI 0.9–1.1). In subjects aged 30–64 years, CD prevalence was 2.4% in Finland (2.0–2.8), 0.3% in Germany (0.1–0.4), and 0.7% in Italy (0.4–1.0). Sixty-eight percent of antibody-positive individuals showed small-bowel mucosal changes typical for CD (Marsh II/III lesion).

Conclusions. CD is common in Europe. CD prevalence shows large unexplained differences in adult age across different European countries.

Key words: *Anti-transglutaminase antibodies, celiac disease, epidemiology, population-based screening, prevalence*

Introduction

Celiac disease (CD) is an autoimmune disorder triggered by gluten, the major protein complex found in wheat, barley, and rye, in genetically predisposed individuals (1). The major problem in diagnosing the disease is its multifaceted clinical picture. Gastrointestinal symptoms may vary from mild to severe or be even totally absent despite the presence of the mucosal lesion. Patients may be clinically silent for years or decades or present only extraintestinal complications, such as osteoporosis, dental enamel defects, or peripheral or central nervous system involvement (2).

Serum IgA class tissue transglutaminase (tTG) antibody enzyme-linked immunosorbent assay

Correspondence: Professor Carlo Catassi, Department of Pediatrics, Università Politecnica delle Marche, Via Corridoni 11, 60123 Ancona, Italy. Fax: +39 071 36281. E-mail: catassi@tin.it

(Received 10 March 2009; accepted 30 June 2010)

ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.3109/07853890.2010.505931

Key messages

- Celiac disease is a common disorder affecting 1% of the European population.
- Celiac disease is underdiagnosed even in European countries with high knowledge of the variable clinical picture of the disease.
- The prevalence of celiac disease varies markedly in different European countries.

Abbreviations

AU	arbitrary unit
CD	celiac disease
ELISA	enzyme-linked immunosorbent assay
EMA	endomysial antibodies
KORA	Co-operative Health Research in the Region of Augsburg
MONICA	multinational monitoring of trends and determinants in cardiovascular diseases
tTG	tissue transglutaminase

(ELISA) test has proved to be a sensitive tool for CD detection, even in atypical or silent cases (3–6). It is an accurate, observer-independent alternative to serum IgA endomysial antibodies (EMA) detected by an indirect immunofluorescence reaction, which is a time-consuming and operator-dependent testing method. IgA class anti-tTG determination is currently considered as the best first-step, CD screening test in the general population (7–10). However, the positive predictive value of tTG is not very high, particularly when the pre-test probability of the disease is low, as in the screening of the general population (9,11). Serum IgA-EMA is a more specific marker of untreated CD (8,9). This autoantibody also predicts the future development of mucosal lesions in individuals with normal small-bowel mucosal morphology (12–16). Therefore the serial determination of IgA-tTG and IgA-EMA (in anti-tTG-positive cases) is a powerful diagnostic algorithm for detecting CD, in terms of both sensitivity and specificity (11,12,17,18).

Despite these advances in CD detection, the overall prevalence of the disease in Europe is still unclear. Early prevalence figures were based on clinical diagnoses, which relied upon the occurrence of gastrointestinal symptoms and malabsorption. At that time many countries reported prevalence figures as low as 0.1% or less (19). Recent studies using serological tests as the screening tool indicate that CD is highly underdiagnosed all over the world, with prevalence figures reaching 0.5%–1.0% or even more (6,7,15, 20–28). However, studies report considerable variation of the occurrence of the disease, both within and between different European countries. Since the sample sizes were often small as compared to the expected prevalence, the statistical power of available studies is limited. For this reason it is not clear whether different estimates reflect by-chance variability or 'true' regional variation of disease prevalence. It is worth noting that a precise estimate of the European prevalence of CD is important not only for epidemiological reasons but also for other aspects, e.g. insurance, agricultural, international trade, and regulatory issues within the European Union.

The aims of the present study were: 1) to establish accurately the prevalence of CD in a large sample of the general European population, including both children and adults, using a centralized serological screening approach; and 2) to investigate whether the prevalence of CD significantly varies between different areas of the European continent. We present here the final results of this study, which was performed on the largest population sample cross-sectionally screened for CD in the world (n=29,212) by the serial determination of IgA-tTG and IgA-EMA in anti-tTG-positive cases.

Materials and methods

Study populations

Sera from 29,212 individuals, both adults and children, from four European countries were sampled for serological studies (Table I). All sera were stored at −20°C/−70°C before serological testing. As indicated below country by country, a proportion of these sera were collected within the frame of other studies (e.g. the MONICA study), but none of them had already been investigated for CD serology.

Finland. A country-wide cross-sectional sample of 6,403 Finnish adults (30–93 years of age) representative of the Finnish urban and rural adult population (3,279,772 inhabitants of this age group) was collected by two-stage cluster sampling as part of the Health 2000 survey in 2000–2001 co-ordinated by the National Public Health Institute of Finland (29). Eligible population was 8,028 and participation rate 80%.

Germany. Two different cross-sectional samples were drawn from the population of Augsburg and two surrounding counties (349,050 inhabitants of age group 25–74 years in 1990 and 357,627 inhabitants in 2001) by sex-age stratified two-stage cluster sampling (30,31).

1) A sample of 4,940 German adults (25–74 years of age) collected for the MONICA (multinational

Table I. Summary of the study results in the overall sample. Prevalence of CD with 95% confidence interval (CI) is reported, based on different criteria: A = Previously diagnosed biopsy-proven CD patients + screening-detected tTG-positive cases; and B = Previously diagnosed biopsy-proven CD patients + cases with both tTG and EMA positivity.

Country, population (collection time)	Sample size *n*/females (%)	Previously diagnosed CD *n*	tTG + *n*	tTG borderline but EMA+*n*	EMA + *n*	Individuals that agreed to a biopsy *n* (%)	Biopsy + *n*	Prevalence A % (95% CI)	Prevalence B % (95% CI)
Finland									
Adults (2000–2001)	6,403/3,527 (55)	38	120	3	87	63 (51)	47	2.5 (2.1–2.9)	2.0 (1.7–2.3)
Germany									
Adults (1989–1990)	4,633/2,300 (50)	0	63	0	7	3 (5)	1	1.4 (1.1–1.7)	0.2 (0.1–0.3)
Adults (1999–2001)	4,173/2,110 (51)	1	18	0	10	7 (39)	5	0.5 (0.3–0.7)	0.3 (0.1–0.5)
Italy									
Adults (2000–2002)	4,781/2,716 (57)	1	65	0	32	42 (65)	23	1.4 (1.1–1.7)	0.7 (0.5–0.9)
Children (1997–2002)	2,645/1,376 (52)	0	33	2	29	27 (77)	19	1.3 (0.9–1.7)	1.1 (0.7–1.5)
UK									
Adults (1986–1987)	4,656/2,306 (50)	13	74	1	55	3 (4)	3	1.9 (1.5–2.3)	1.5 (1.1–1.9)
Children (2000)	1,975/973 (49)	1	18	4	12	2 (9)	2	1.0 (0.6–1.4)	0.9 (0.5–1.3)
Total	29,266/15,308 (52)	54	391	10	232	147 (38)	100	1.5 (1.4–1.7)	1.0 (0.9–1.1)
Adults	24,646/12,959 (53)	53	340	4	191	118 (35)	79	1.6 (1.4–1.8)	1.0 (0.9–1.1)
Children	4,620/2,349 (51)	1	51	6	41	29 (57)	21	1.1 (0.8–1.4)	0.9 (0.6–1.2)

CD = celiac disease; tTG = tissue transglutaminase antibodies; EMA = endomysial antibodies.

monitoring of trends and determinants in cardiovascular diseases) Augsburg population-based survey in 1989–1990 (30). Eligible population was 6,420 and participation rate 76.9%. Participants who refused to give blood or participants without enough serum for analysis were excluded (n=307; 4.7%) leaving 4,633 participants for the analysis presented here. Follow-up data from anti-tTG-positive cases included in this subgroup of 4,633 participants have been described (31).

2) A sample of 4,261 German adults (25–74 years of age) collected for the KORA (Co-operative Health Research in the Region of Augsburg) population-based survey in 1999–2001 (32). Eligible population was 6,417 and participation rate 66.4%. Participants who refused to give blood or participants without enough serum for analysis were excluded (n=88; 1.4%), leaving 4,173 participants for the analysis presented here.

Italy. A cross-sectional sample of 4,778 adults (20–100 years of age) and 2,649 children (0–16 years of age) was collected in three different surveys including all inhabitants of the village of Camerano (Ancona) in 2000–2002, inhabitants from the village Uri (Sassari) in 1999, and a school-child population aged 10–19 years attending the secondary school in Alghero (Sassari) in 1997–1999. Eligible population was altogether 11,978 and participation rate 62%.

UK. The study population of the UK also consisted of two different samples:

1) Sera of 4,656 adults (25–64 years of age) from Belfast and surrounding district (223,575 inhabitants of this age group) were provided for this study from the MONICA 2 survey in 1986–1987 (33). Eligible population was 4,863 and participation rate 96%.

2) A sample of 1,975 children aged 12 and 15 years participating the Northern Ireland Younghearts 2000 Survey of risk factors for cardiovascular disease was collected in 2000. Eligible population was 2,017 and participation rate 98%.

The details about the material collections have been published elsewhere (22,24).

Study protocol

The overall study protocol is described in Figure 1. Previously diagnosed CD cases, as well as individuals on a gluten-free diet for other reasons than CD, were identified by structured questionnaires, as a part of the original health surveys, and excluded from serological CD screening.

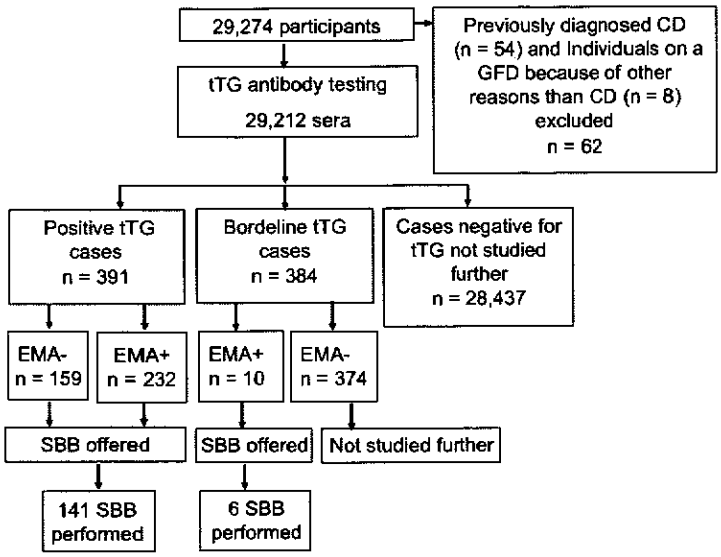

Figure 1. Study design. tTG = tissue transglutaminase; CD = celiac disease; GFD = gluten-free diet; EMA = endomysial antibodies; SBB = small-bowel biopsy.

Serum tissue transglutaminase antibody testing

All 29,212 sera were tested blindly for IgA class tTG antibodies in the laboratory of Eurospital, Trieste, Italy, using a human recombinant tTG-based antibody kit (Eu-tTG®system, Eurospital, Trieste, Italy). Validation by the manufacturer showed the sensitivity of the kit in detecting EMA-positive sera to be 98.2% and the specificity 97.2%. The sensitivity of the kit in detecting biopsy-proven celiac disease was 96.3% and the specificity 84.7%. Cut-off values were assigned as <7.0 AU/mL for negative results and ≥7.0 AU/mL for positives. In the present study titers between 5 and 6.9 AU/mL were considered border-line and were further tested for EMA.

Serum endomysial antibody testing

All tTG antibody-positive (≥7.0 AU/mL) as well as border-line titer sera (5–6.9 AU/mL) were further tested for the presence of IgA-EMA with an indirect immunofluorescence (Antiendomysium®, Eurospital SpA, Trieste, Italy) performed by Eurospital, Trieste, Italy. A patient serum dilution titer of 1:≥5 was considered positive.

Small-bowel biopsies

Confirmation of the CD diagnosis by small-bowel biopsy was recommended to all anti-tTG antibody-positive individuals and to individuals showing border-line anti-tTG antibodies associated with EMA positivity. Gluten exposure was ascertained at the time of the intestinal biopsy. Formalin-fixed biopsy specimens (at least four specimens from both duodenal bulb and first duodenal tract) were stained with hematoxylin and eosin. All biopsies were examined in a single center (Ancona) by a pathologist (I.B. and A.M.) who was not aware of the antibody test results. According to the Marsh classification (34) the histological damage was graded as I (infiltrative lesion), II (infiltrative-hyperplasic lesion), or III (partial or subtotal villous atrophy with crypt hyperplasia).

All patients newly diagnosed with CD were prescribed a gluten-free diet.

Case definition

In this study we report two different CD-related prevalence figures:

1) Prevalence A: Previously diagnosed biopsy-proven (Marsh II or III) CD patients + screening-detected anti-tTG-positive individuals.

2) Prevalence B: Previously diagnosed biopsy-proven (Marsh II or III) CD patients + screening-detected individuals with both anti-tTG and EMA positivity.

Statistical analyses

Ninety-five percent confidence intervals (CI) for the prevalence figures obtained in different materials were calculated as $1.96 \times \sqrt{\frac{p(1-p)}{N}}$ where p is the proportion of positivity in the population and N is the sample size.

Ethical aspects

The participants gave their written consent for the study. Local national ethical approval was obtained by all centers involved in the study. The Helsinki Declaration was respected throughout the study. All records and other information of the persons were stored according to the EU and national regulations.

Results

Antibody positivity

The data of antibody positivity, biopsy rates and results, and prevalence figures are shown in Table I. In all materials there was somewhat more tTG positivity than EMA positivity. In all countries serum antibody screening detected a number of previously undiagnosed cases.

Small-bowel biopsies

Of all 401 autoantibody-positive individuals recommended for small-bowel biopsy, 147 (37%) agreed. Altogether 28 individuals had died after serum sampling, 128 refused, 49 could not be traced, and in 49 cases the local health care system or the family doctor did not find the small-bowel biopsy necessary. In 100/147 (68%) biopsied persons the histological analysis showed a typical CD enteropathy (grade II or III Marsh lesion). Ninety-one of these were both tTG and EMA antibody-positive, while nine were anti-tTG antibody-positive but EMA-negative. Twenty-five individuals showed minor changes in their small-bowel mucosal specimen (Marsh I lesion). Fourteen of them were positive for both anti-tTG and EMA antibodies, and 11 were positive for tTG but negative for EMA antibodies. Only 16 persons (11%) did not have any inflammatory changes in their small-bowel mucosal specimen. Eight of these had both antibody tests positivity, and eight were positive for tTG but negative for EMA antibodies. Six specimens (4%) were not valid for histological inspection.

CD prevalence in Europe

The prevalence of CD in the overall sample ($n=29,266$) was 1.5% (prevalence A) and 1% (prevalence B), with a very narrow confidence interval (1.4–1.7 for prevalence A and 0.9–1.1 for prevalence B, respectively). In all samples the prevalence figures obtained by anti-tTG antibody positivity alone (prevalence A) were higher than those based on combined anti-tTG and EMA antibody positivity (prevalence B). Prevalence A was higher in adults than in children (1.6 versus 1.1), while prevalence B was similar in both groups (1.0 in adults and 0.9 in children, respectively). The prevalence of CD varied widely across countries, with the highest CD prevalence being found in Finnish adults (prevalence A=2.5 and prevalence B=2.0), and the lowest in one of the two German samples (prevalence A=0.5 and prevalence B=0.3) (Table I).

Since the population samples from different countries were not comparable for age range and time of sampling, we then analyzed CD prevalence in the homogeneous subsample including only adults aged 30–64 years investigated during a short time-span (1999–2002), to allow direct comparison of CD prevalence between different European countries. The prevalence estimates in this subgroup ($n=10,703$) largely overlapped with those reported on the overall sample: overall prevalence A was 1.4 (95% CI 1.2–1.6), and overall prevalence B was 1.0 (0.8–1.2). Again, a large inter-country variability was found, with a 5–8-fold difference between the country showing the highest CD prevalence (Finland, prevalence A=2.6% and prevalence B=2.4%) and the country showing the lowest CD prevalence (Germany, prevalence A=0.5% and prevalence B=0.3%) (Table II).

The size of the celiac iceberg in different European countries

In the subgroup reported in Table II, the overall ratio between previously diagnosed CD cases ($n=29$) and the prevalence of CD (previously diagnosed + EMA positives, $n=139$) was 0.21 (1 in 5). However this proportion varied markedly between countries (Finland=0.24; Germany=0.12; Italy=0.06).

Discussion

On a large population sample this study definitely confirms that CD is one of the commonest lifelong disorders in Europe, affecting around 1% of the general population in both children and adults. This estimate is now more accurate than previously available data, for the following reasons: 1) it is the largest population sample ($n=29,266$) so far investigated in the world. Only two CD screening studies had previously been conducted on more than 10,000 subjects of the general population, one in Italy ($n=17,201$) (35) and the other in the US ($n=12,678$) (36); and 2) this is the first study to be performed in different European countries using the same diagnostic algorithm and centrally performed determinations. We used the observer-independent serum IgA-tTG test based on human recombinant tTG for the first-level screening of CD because the test is

Table II. Results of CD screening in adults aged 30 to 64 years investigated over a restricted time-span. Prevalence of CD with 95% confidence interval (CI) is reported, based on different criteria: A = Previously diagnosed biopsy-proven CD patients + screening-detected tTG-positive cases; and B = Previously diagnosed biopsy-proven CD patients + cases with both anti-tTG and EMA positivity.

	Sample size *n*/females (%)	Previously diagnosed CD *n*	tTG + *n*	EMA + *n*	tTG border-line – but EMA + *n*	Individuals that agreed to a biopsy *n* (%)	Biopsy + *n*	Prevalence A % (95 % CI)	Prevalence B % (95 % CI)
Finland (2000–2001)	4,846/2,548 (53)	27	100	83	3	55 (53)	40	2.6 (2.2–3.1)	2.4 (2.0–2.8)
Germany (1999–2001)	3,098/1,574 (51)	1	14	7	0	6 (43)	0	0.5 (0.2–0.7)	0.3 (0.1–0.4)
Italy (2000–2002)	2,759/1,606 (58)	1	31	17	0	25 (81)	12	1.2 (0.8–1.6)	0.7 (0.4–1.0)
Total	10,703/5,728 (54)	29	145	107	3	86 (59)	52	1.4 (1.2–1.6)	1.0 (0.8–1.2)

CD = celiac disease; tTG = transglutaminase antibodies; EMA = endomysial antibodies.

accurate and easy to perform and it has shown high sensitivity in detecting untreated CD (3–10). European ring testing of CD-specific autoantibodies has shown differences in the results of antibody tests between different laboratories (37). Even minimal differences in the test performance may cause large differences in the number of subjects that have a positive test when screening the general population. Therefore we centralized the antibodies determinations, and all 29,212 sera were tested in the same laboratory. To increase the specificity of the diagnostic procedure we performed also the EMA test in anti-tTG-positive or border-line samples, because there is evidence in the literature suggesting a higher positive predictive value of the EMA compared with the tTG test, particularly when the tTG titer is only modestly increased (9,11).

Not only is CD very common in Europe, but it is also highly underdiagnosed in all countries—even in countries where there is traditionally a high knowledge of the disease (e.g. Finland). In our adult subsample we found that the ratio between the 'visible' (previously diagnosed) and the overall size (overall prevalence) of the celiac iceberg varied between 6% (Italy) and 24% (Finland). These results have implications affecting the European society from several points of view. Since untreated CD is associated with a number of complications (e.g. infertility and cancer) (1), the first issue is how the diagnostic rate of this common disorder can be improved. The pros and cons of either CD mass screening or case finding have been recently reviewed (38). Case finding in at-risk individuals (e.g. subjects with anemia, chronic digestive complaints, or family history of CD) seen at the primary care level is a cheap and ethically sound approach to CD detection. In the US a case finding approach in the primary care setting increased the rate of diagnosis by 32- to 43-fold (39). However, the sensitivity of a case finding policy is low, as more than 50% are left undiagnosed (40). Although CD meets most of the WHO criteria for mass screening programs (41), controversies on diagnosis, poor understanding of the natural course of undiagnosed CD, and difficulties with the adherence to a gluten-free diet all suggest caution before implementation of mass screening programs. A recent Markov model-based analysis showed that mass screening for CD of the young adult general population is associated with improved quality-adjusted life years (QALY) and becomes a cost-effective strategy if the time delay to diagnosis is longer than 6 years (42). Whatever diagnostic strategy will eventually be shown to be superior, our data clearly indicate that this common food intolerance deserves a high level of awareness in Europe because of its health, nutritional, social, psychological, and economic implications.

Using homogeneous diagnostic criteria in adults aged 30–64 years, this population-based screening study showed that the prevalence of CD in the adult population varied markedly and significantly in different European countries, with the highest prevalence being found in Finland (2.4%) and the lowest in Germany (0.3%) (Table II). The reasons for these large between-country differences are not clear but could be related to both genetic and environmental factors, such as the dietary pattern during infancy. Recent studies have shown that nutritional factors such as the duration of breast-feeding, age at gluten introduction, and amount of gluten introduced during the first year of life can influence not only the clinical presentation of the disease but also the overall prevalence of CD (25). Interestingly, no South-North geographical gradient was noted, as an intermediate CD prevalence was found in the Southern country participating to this project (Italy, 0.7%). Then our results question the validity of an old theory relating CD prevalence in Europe to past agriculture spreading. According to this model, the slow spreading of gluten-rich cereals culture and consumption from the so-called 'fertile crescent' area in the Near East exerted a negative selective pressure on CD-predisposing genes during the last ten thousand years (43). Higher CD prevalence should therefore be found in North European countries, where gluten-rich cereals have been introduced more recently into the daily diet. This hypothesis was not confirmed by our data, as we found that the country reached earlier by agriculture practices (Italy) showed higher CD prevalence than Germany, an area colonized by farmers later on.

It is important to note that the case definition influences prevalence estimates in epidemiological studies. Whatever case definition is preferred, there is a risk of patient misclassification. This issue is particularly important for CD, a widely variable disorder including cases with positive serology and normal histology (potential CD) (13) and cases with negative serology and positive histology (seronegative CD) (44). Although the small-intestinal biopsy has an indisputable diagnostic role in the clinical setting, this invasive investigation is not an essential requirement for an epidemiological survey. As a matter of fact, in many recently published epidemiological studies the antibody positivity has been considered as the only criterion for CD diagnosis (22–24). A CD prevalence study based on serological screening presents factors that could lead to slight over- or under-estimation of CD prevalence. Since the sensitivity of serology is lower in patients showing a modest degree of intestinal damage (e.g. Marsh I lesion) (44), serological screening may under-estimate the 'true' prevalence. On the other hand, there are patients with positive serology and normal histology. Some of these could be true false positives and will lead to a possible over-estimation of CD prevalence, while the remainder may have true disease missed on biopsy (potential CD). This level of uncertainty mostly applies to our prevalence A, which we consider less accurate than prevalence B, because many subjects showed isolated and modestly increased anti-tTG positivity. Conversely, the possibility of a false positivity of both tTG and EMA (not evolving in overt CD with time) is still to be clearly documented in the literature. In our study antibody-positive individuals were offered the opportunity for a small-bowel biopsy. The biopsy rate varied markedly between different centers (from 77% in Italy to 9% in the UK) mostly due to differences in the study populations. Especially in the old materials many participants were untraceable, e.g. because of change of address or death. Among traceable participants the main reasons for low biopsy rate was that either the family doctor did not find the biopsy necessary or participants refused the diagnostic procedure. Altogether 68% of biopsied autoantibody-positive participants showed small-bowel mucosal changes typical for CD (Marsh II or III lesion). Based on our histological findings, the previously reported risk of patients' misclassification applies to a maximum of 20% of patients (the 9/141 with isolated anti-tTG positivity and Marsh II or III lesion, the 11/141 with isolated anti-tTG positivity and Marsh I lesion, plus the 8/141 with anti-tTG positivity and normal histology).

We are aware of the possible limitations of this study: 1) With the exception of Finland, the sampling criteria were not homogeneous for geographic and age distribution of sampled individuals. There was under-representation of children in some countries. For this reason we restricted between-country comparisons of CD prevalence to a well defined age range (30–64 years). 2) The occasional patient with IgA deficiency and CD would have been missed by our IgA-based anti-tTG and EMA screening algorithm. 3) The MONICA materials from Germany and the UK were collected earlier than other sera. This could influence our overall epidemiological findings reported in Table I, as it has been reported that CD prevalence could change (increase) over time (36,45). 4) The number of previously diagnosed CD cases was identified by questionnaires as a part of the original health surveys. Since there could be an ascertainment bias in MONICA individuals investigated years before, we restricted the analysis of the diagnosed/undiagnosed ratio (celiac iceberg) to the *de novo* investigated subjects (Table II). And 5) the ratio between anti-tTG and EMA positivity was around 1.5 in most national samples,

suggesting the possible 'false' positivity of low-titer anti-tTG. However in the German material collected in 1989–1990 the number of anti-tTG-positive sera was ten times higher than EMA (Table I), for reasons that remain unclear. We hypothesize that these old German samples underwent dehydration during storage affecting the tTG quantitative determination but not the semi-quantitative EMA test performed by indirect immunofluorescence. In the end, this outlier result had poor influence on CD prevalence B shown in Table I (including only EMA-positive cases) and none on the data presented in Table II.

In conclusion, this study definitely confirms that CD is one of the commonest lifelong disorders affecting around 1% of the European population. The disease is still heavily underdiagnosed in all European countries. Quite surprisingly, we found a huge difference in CD prevalence between European countries (8-fold in our samples). Environmental factors responsible for the wide variability of CD prevalence between European countries need further investigation, as this knowledge could pave the way to primary prevention of this lifelong disorder.

Acknowledgements

This work was supported by the Commission of the European Communities, the specific RTD program 'Quality of Life and Management of Living Resources', QLRT-1999-00037, 'Evaluation of the prevalence of celiac disease and its genetic components in the European population'. The study does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area. Further, this study was financially supported by Research Council for Health, Academy of Finland.

Kirsi Mustalahti and Carlo Catassi contributed equally to this paper.

Co-authors and members of the Coeliac EU Cluster, Project Epidemiology: Kaija Laurila[a], MSc, Pekka Collin[b], MD, PhD, Katri Kaukinen[a,b], MD, PhD, Vesa Tanskanen[c], MSc, Harri Rissanen[c], MSc, Alun Evans[d], MD, Peter Watson[d], MD, Giuseppe Fanciulli[e], MD, Italo Bearzi[f], MD, Alessandra Mandolesi[f], MD, H.-Erich Wichmann[g], MD, PhD.

Affiliations: [a]Paediatric Research Centre, University of Tampere, Tampere, Finland, [b]Department of Gastroenterology, Tampere University Hospital, Tampere, Finland, [c]National Public Health Institute, Helsinki, Finland, [d]Department of Epidemiology and Public Health, Queens University Belfast, Belfast, UK, [e]Department of Pediatrics, General Hospital, Alghero, Sassari, Italy, [f]Department of Pathology, Università Politecnica delle Marche, Ancona, Italy, [g]Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Germany

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

Maurizio Gasparin and Enzo Bravi are employees of Eurospital SpA, Trieste, Italy. They did not receive any financial support or other credits for this work.

References

1. Green PH, Jabri B. Coeliac disease. Lancet. 2003;362: 383–91.
2. Collin P, Kaukinen K, Välimäki M, Salmi J. Endocrinological disorders and celiac disease. Endocr Rev. 2002;23: 464–83.
3. Dieterich W, Laag E, Schöpper H, Volta U, Ferguson A, Gillett H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease. Gastroenterology. 1998;115: 1317–21.
4. Bürgin-Wolff A, Dahlbom I, Hadziselimovic F, petersson CJ. Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. Scand J Gastroenterol. 2002;37:685–91.
5. Van-Meensel B, Hiele M, Hoffman I, Vermeire S, Rutgeerts P, Geboes K, et al. Diagnsotic accuracy of ten second-generation (human) tissue transglutaminase antibody assays in celiac disease. Clin Chem. 2004;50:2125–35.
6. Fabiani E, Peruzzi E, Mandolesi A, Garbuglia G, Fanciulli G, D'Appello AR, et al. anti-human versus anti-guinea pig tissue transglutaminase antibodies as the first-level serological screening test for coeliac disease in the general population. Dig Liver Dis. 2004;36:671–6.
7. Akbari MR, Mohammadkhani A, Fakheri H, Javad Zahedi M, Shahbazkhani B, Nouraie M, et al. Screening of the adult population in Iran for coeliac disease: comparison of the tissue-transglutaminase antibody and anti-endomysial antibody test. Eur J Gastroenterol Hepatol. 2006;18: 1181–6.
8. Biagi F, Pezzimenti D, Campanella J, Vadacca GB, Corazza GR. Endomysial and tissue transglutaminase antibodies in coeliac sera: a comparison not influenced by previous serological testing. Scand J Gastroenterol. 2001;36:955–8.
9. Lock RJ, Stevens S, Pitcher MC, Unsworth DJ. Is immunoglobulin A anti-tissue transglutaminase antibody a reliable serological marker of coeliac disease? Eur J Gastroenterol Hepatol. 2004;16:467–70.
10. Hopper AD, Hadjivassiliou M, Huristone DP, Lobo AJ, McAlindon ME, Egner W, et al. What is the role of serologic testing in celiac disease? A prospective, biopsy confirmed study with economic analysis. Clin Gastroenterol Hepatol. 2008;6:314–20.
11. Bardella MT, Trovato C, Cesana BM, Pagliari C, Peracchi M. serological markers for coeliac disease: is it time to change? Dig Liver Dis. 2001;33:426–31.
12. Collin P, Helin H, Mäki M, Hällström O, Karvonen A-L. Follow-up of patients positive in reticulin and gliadin antibody tests with normal small bowel biopsy findings. Scand J Gastroenterol. 1993;28:595–8.

13. Troncone R. Latent coeliac disease in Italy. Acta Paediatr. 1995;84:1252–7.
14. Kaukinen K, Mäki M, Partanen J, Sievänen H, Collin P. Celiac disease without villous atrophy: revision of criteria called for. Dig Dis Sci. 2001;46:879–87.
15. Mäki M, Mustalahti K, Kokkonen J, Kulmala P, Haapalahti M, Karttunen T, et al. Prevalence of celiac disease among children in Finland. N Engl J Med. 2003;348:2517–24.
16. Salmi TT, Collin P, Järvinen O, Haimila K, Partanen J, Laurila K, et al. Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. Aliment Pharmacol Ther. 2006;24:541–52.
17. West J, Lloyd CA, Hill PG, Holmes GK. IgA-antitissue transglutaminase: validation of a commercial assay for diagnosing coeliac disease. Clin Lab. 2002;48:241–6.
18. Walker MM, Murray JA, Ronkainen J, Aro P, Storskrubb T, D'Amato M, et al. Detection of celiac disease and lymphocytic enteropathy by parallel serology and histopathology in a population-based study. Gastroenterology. 2010;139:112–9.
19. Greco L, Mäki M, Di Donato F, Visakorpi JK. Epidemiology of coeliac disease in Europe and the Mediterranean area. A summary report on the multicentre study by the European Society of Paediatric Gastroenterology and Nutrition. In: Auricchio S, Visakorpi JK, editors. Common food intolerances 1: Epidemiology of coeliac disease. Dynamic Nutrition Research vol. 2. Basel: Karger; 1992. p. 25–44.
20. Catassi C, Rätsch IM, Fabiani E, Rossini M, Bordicchia F, Candela F, et al. Celiac disease in the year 2000: exploring the iceberg. Lancet. 1994;343:200–3.
21. Csizmadia C, Mearin M, von Blomberg B, Brand R, Verloove-Vanhorick S. An iceberg of childhood coeliac disease in the Netherlands. Lancet. 1999;353:813–4.
22. West J, Logan RF, Hill PG, Lloyd A, Lewis S, Hubbard R, et al. Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. Gut. 2003;52:960–5.
23. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 2003;163:286–92.
24. Bingley PJ, Williams AJ, Norcross AJ, Unsworth DJ, Lock RJ, Ness AR, et al. Undiagnosed coeliac disease at age seven: population based prospective birth cohort study. BMJ. 2004;328:322–3.
25. Carlsson A, Agardh D, Borulf S, Grodzinsky E, Axelsson I, Ivarsson A. Prevalence of celiac disease: before and after a national change in feeding recommendations. Scand J Gastroenterol. 2006;41:553–8.
26. Dube C, Rostom A, Sy R, Saloojee N, Garritty C, Sampson M, et al. The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review. Gastroenterology. 2005;128:S57–67.
27. Henker J, Losel A, Conrad K, Hirsch T, Leupold W. Prevalence of asymptomatic coeliac disease in children and adults in Dresden region of Germany. Dtsch Med Wochenschr. 2002;127:511–5.
28. Johnston SD, Watson RG, McMillan SA, Sloan J, Love AH. Prevalence of coeliac disease in Northern Ireland. Lancet. 1997;350:1370.
29. Aromaa A, Koskinen S, editors. Health and functional capacity in Finland. Baseline results of the Health 2000 health examination survey. Publications of the National Public Health Institute B12/2004. Helsinki 2004. Available at: www.ktl.fi/health2000. (accessed: September 14, 2004.)
30. Hense H-W, Filipiak B, Doering A. Ten-year trends of cardiovascular risk factors in the MONICA Augsburg region in Southern Germany. Results from the 1984/1985, 1989/1990, and 1994/1995 surveys. CVD Prevention. 1998;1:318–27.
31. Metzger MH, Heier M, Mäki M, Bravi E, Schneider A, Löwel H, et al. Mortality excess in individuals with elevated IgA anti-transglutaminase antibodies: the KORA/MONICA Augsburg cohort study 1989–1998. Eur J Epidemiol. 2006; 21:359–65.
32. Holle R, Happich M, Lowel H, Wichmann HE. KORA—a research platform for population based health research. Gesundheitswesen. 2005;67 Suppl 1:S19–25.
33. Patterson CC, McCrum EE, Mathewson Z, Evans AE. Validation of two methods of long-term epidemiological follow-up. Ulster Med J. 1990;59:177–82.
34. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology. 1992;102:330–54.
35. Catassi C, Fabiani E, Rätsch IM, Coppa GV, Giorgi PL, Pierdomenico R, et al. The coeliac iceberg in Italy. A multicentre antigliadin antibodies screening for coeliac disease in school-age subjects. Acta Paediatr Suppl. 1996;412:29–35.
36. Rubio-Tapia A, Kyle RA, Kaplan ER, Johnson DR, Page W, Erdtmann F, et al. Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology. 2009;137:88–93.
37. Stern M. Comparative evaluation of serologic tests for celiac disease: A European initiative toward standardization. J Pediatr Gastroenterol Nutr. 2000;31:513–9.
38. Fasano A, Evans K. Should we screen for coeliac disease? BMJ. 2009;339:998–9.
39. Catassi C, Kryszak D, Louis-Jacques O, Duerksen DR, Hill I, Crowe SE, et al. Detection of celiac disease in primary care: a multicenter, case-finding study in North America. Am J Gastroenterol. 2007;102:1454–60.
40. Berti I, Della Vedova R, Paduano R, Devetta M, Caradonna M, Villanacci V, et al. Coeliac disease in primary care: evaluation of a case-finding strategy. Dig Liver Dis. 2006; 38:461–7.
41. Fasano A. Should we screen for celiac disease? Yes. BMJ. 2009;339:b3592.
42. Hershcovici T, Leshno M, Goldin E, Shamir R, Israeli E. Cost effectiveness of mass screening for coeliac disease is determined by time-delay to diagnosis and quality of life on a gluten free diet. Aliment Pharmacol Ther. 2010;31:901–10.
43. Cronin CC, Shanahan F. Why is celiac disease so common in Ireland? Perspect Biol Med. 2001;44:342–52.
44. Salmi TT, Collin P, Korponay-Szabo IR, Laurila K, Partanen J, Huhtala H, et al. Endomysial antibody-negative celiac disease: clinical characteristics and intestinal autoantibody deposits. Gut. 2006;55:1746–53.
45. Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, et al. Increasing prevalence of celiac disease over time. Aliment Pharmacol Ther. 2007;26:1217–25.