ACTA ABP BIOCHIMICA POLONICA

Vol. 57, No 1/2010
115–118
on-line at: www.actabp.pl

Regular paper

Cystic fibrosis is a risk factor for celiac disease

Jarosław Walkowiak[2]✉, Anna Blask-Osipa[1], Aleksandra Lisowska[1], Beata Oralewska[3], Andrzej Pogorzelski[4], Wojciech Cichy[1], Ewa Sapiejka[5], Mirosława Kowalska[6], Michał Korzon[7] and Anna Szaflarska-Popławska[8]

[1]Department of Gastroenterology & Metabolism, First Chair of Pediatrics, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Dietetics, Chair of Human Nutrition & Hygiene, Poznań University of Life Sciences, Poznań, Poland; [3]Department of Pediatric Gastroenterology, Hepatology & Immunology, Child Memorial Health Institute, Warszawa, Poland; [4]Department of Bronchology & Cystic Fibrosis, National Institute for Tuberculosis & Lung Diseases, Pediatric Branch, Rabka, Poland; [5]Out-Patient Clinic for CF Patients, Gdańsk, Poland; [6]Out-Patient Clinic for Patients with Pulmonary Diseases "Panaceum", Koszalin, Poland; [7]Department of Obstetrics, Medical University of Gdańsk, Gdańsk, Poland; [8]Chair of Pediatrics, Gastroenterology and Alergology, Collegium Medicum Bydgoszcz, Mikołaj Kopernik University, Toruń, Poland

Background: The coexistence of cystic fibrosis (CF) and celiac disease (CD) has been reported. To our knowledge there is no study directly comparing the incidence of CD in CF patients to that in the general population at the same time. There is no published data on genetic predisposition to CD in CF patients either. Therefore, in the present study we aimed to assess the genetic predisposition to CD and its incidence in CF patients comparing it to data from the general population. Patients and methods: Two hundred eighty-two CF patients were enrolled in the study. In 230 CF patients the genetic predisposition to CD (the presence of HLA-DQ2/ DQ8) was assessed. In all CF patients, serological screening for CD was conducted. In patients with positive antiendomysial antibodies (EMA) gastroduenoscopy was offered. Intestinal histology was classified according to modified Marsh criteria. The results of serological CD screening in 3235 Polish schoolchildren and HLA-DQ typing in 200 healthy subjects (HS) were used for comparison. Results: Positive EMA was found in 2.84% of the studied CF patients. The incidence of proven CD was 2.13%. The incidence of CD as well as positive serological screening were significantly more frequent in the CF group than in the general population. The frequency of CD-related HLA-DQ alleles in CF and HS did not differ. Conclusions: Genetic predisposition to celiac disease in cystic fibrosis patients is similar to that of the general population. However, our results suggest that cystic fibrosis is a risk factor for celiac disease development.

Keywords: cystic fibrosis, celiac disease, antiendomysial antibodies, genetic predisposition

Received: 29 December, 2009; revised: 03 March, 2010; accepted: 18 March, 2010; available on-line: 20 March, 2010

INTRODUCTION

Celiac disease (CD) is a chronic autoimmune disease that can appear in genetically predisposed subjects throughout life (Di Sabatino & Corazza, 2009). However, only a minority of the predisposed children and adults develop CD. Results from genetic linkage studies show that the disease is strongly associated with HLA-DQ genes. Most patients carry the DQ2 variant, in the remaining subjects the DQ8 allele has been documented (Sollid *et al.*, 1989). The development of CD is dependent on environmental factors (the ingestion of wheat, barley or rye) with possible participation of other cofactors (infant-feeding practices, intestinal infections, drugs, etc.) The epidemiology, pathophysiology and clinical aspects of CD have been recently extensively reviewed (Di Sabatino & Corazza, 2009).

Cystic fibrosis (CF) and CD were for many years recognized as one clinical entity. Their separation took place in the thirties of the 20th century. In 1999 Venuta and coworkers described a patient suffering from CF and CD and reviewed the available literature summarizing 16 documented cases of CF coexisting with CD (Venuta *et al.*, 1999). Two Veronian reports suggested a higher incidence of CD in the CF population than in healthy subjects (Valletta & Mastella 1989, Pardo 1991). However, the occurrence of CD in the general population at that time was not well known. Based on those results CF was believed to be a risk factor for CD development (Borowitz *et al.*, 2005). Subsequent studies revealed that the CD frequency in the general population was underestimated (Maki *et al.*, 2003; Fasano *et al.*, 2003; West *et al.*, 2003; Tommasini *et al.*, 2004). Therefore, the Veronian incidence of CD in CF patients (Valletta & Mastella, 1989; Pardo, 1991) could simply represent the general incidence of CD. Recently, Fluge *et al.* (2009) reported co-morbidity of CF and CD in six Scandinavian CF centers. They documented a 1.2% prevalence of CD among the studied CF subjects. However, they did not have a reliable control or reference group, which caused a high level of uncertainty. To our knowledge there is no published study directly comparing the incidence of CD among CF patients to that in the general population at the same time. Moreover, there is no published data on the genetic predisposition to CD in CF.

Therefore, in the present study we aimed to assess the genetic predisposition to CD and its incidence in CF patients comparing it to data from the general population.

PATIENTS AND METHODS

The survey was carried out in the years 2006–2009. Two hundred eighty-two CF patients (142 females and

✉e-mail: jarwalk@ump.edu.pl

Abbreviations: CD, celiac disease; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; EMA, antiendomysial antibodies; HLA, human leukocyte antigens; HS, healthy subjects; Ig, immunoglobine; PCR, polymerase chain reaction; SSOP, sequence specific oligonucleotide probes; tTG, tissue transglutaminase antibodies.

140 males) aged from 3 to 42 years (median age 12 years) enrolled in the study. The exclusion criteria were age below three years (limited sensitivity of serological methods in this age group). Diagnosis of CF was based on history, clinical manifestation and increased sweat chloride concentrations and confirmed by the *CFTR* gene analysis.

In 230 CF patients the genetic predisposition to CD was assessed (Biotest, Dreieich, Germany). HLA-DQ typing was performed by DNA amplification by the polymerase chain reaction (PCR) and hybridization with sequence specific oligonucleotide probes (SSOP) (Bignon, 1997).

In all the CF subjects, serological screening for CD was conducted. Tissue transglutaminase in the IgA subclass (tTG IgA; Immundiagnostik, Germany) was measured (Bossuyt & Blanckaert, 1999). In five patients with low IgA concentrations (below the lower limit of the age range), tTG IgG was additionally determined (Dietrich *et al.*, 1997). In subjects with abnormal (high) tTG concentrations, antiendomysial antibodies in IgA subclass (EMA IgA) were searched for (Chorzelski *et al.*, 1984). In the EMA-positive patients the gastroduenoscopy (with duodenal biopsy) was offered. At least three samples from different regions of the duodenum were taken. Intestinal histology was classified according to modified Marsh criteria (Marsh, 1992; Oberhuber *et al.*, 1999).

The results of serological CD screening performed in the group of 3235 Polish schoolchildren aged 11 years served as a reference value of CD occurrence in the general Polish population. In all subjects IgA and IgG EMA were measured. Small bowel endoscopic biopsy was proposed in cases of positive EMA IgA or IgG (Szaflarska-Popławska *et al.*, 2009). Two hundred healthy subjects (HS) aged 18 to 25 years created the control group in which HLA-DQ typing was performed.

The differences in the prevalence of the HLA-DQ2/DQ8 alleles and the CD occurrence in the CF and general populations were assessed with the use of chi^2 test. The level of significance was set at $P<0.05$. The protocol of the investigation was approved by the Ethical Committee of the Poznań University of Medical Sciences (Poland).

RESULTS

In two (0.7%) CF patients introduced into the study CD diagnosis was established earlier (positive antibodies and total villous atrophy). Positive IgA tTG was documented in 11 (3.9%) subjects. HLA-DQ study confirmed genetic predisposition to CD in this subgroup. In five patients with low IgA concentrations IgG tTG was not detected. Positive titres of EMA IgA were found in 6 out of 11 preselected patients (tTG positive). The consent for small intestine biopsy was obtained from five patients with both positive antibodies and from four with positive tTG and negative EMA. Four out of these nine patients were found to have CD (Table 1).

Table 1. Basic data of children with positive serological tests

Patient	tTG IgA (RU/ml)	EMA IgA	Marsh classification	HLA-DQ
P1	97	–	N.a.	2
P2	158	–	0	2
P3	262	+	3c	2
P4	76	–	0	2
P5	54	+	0	N.a.
P6	235	+	3c	2, 8
P7	70	–	0	8
P8	97	–	0	8
P9	256	+	N.a.	2
P10	>270	+	3c	8
P11	>270	+	3b	2

N.a., not available (lack of consent at the moment of study)

Summing up the data, the presence of positive tTG was found in 13 (4.61%) and EMA in eight (2.84%) of the studied CF patients. The incidence of proven CD was 2.13%. The adjusted values resulting from the extrapolation of positive results of biopsies in the endoscoped patients into those who did not consent (calculating patients with both positive antibodies) would be higher. Eleven out of 25 positive non-CF schoolchildren underwent small bowel biopsy. Seven of them had abnormal mucosal architecture. In one girl CD had been diagnosed earlier. The proven CD incidence was 0.25%. The parents of the remaining 14 children either did not come for subsequent visit or refused the gastroduodenoscopy to be performed in their children (Szaflarska-Popławska *et al.*, 2009). The incidence of CD as well as positive results of serological screening were significantly more frequent in the studied CF population than in the general population (Table 2). However, the frequency of CD-related HLA-DQ alleles in CF and HS did not differ (Table 3).

DISCUSSION

A significantly higher prevalence of celiac disease among Polish CF patients than in healthy subjects is reported in the present study. To our knowledge this is the first study directly comparing the incidence of celiac disease in the CF and general populations in a reliable way.

The majority of CD symptoms may appear as a gastrointestinal manifestation of CF. It was undoubtedly the reason of the difficulties in separation of both clinical entities in the past. At present, clinical manifesta-

Table 2. Frequency of celiac disease and positive serological screening in CF patients and general population

		Population percentage		Statistical significance
		CF	HS**	
Positive serological screening (%)*		2.84	0.80	0.0001
CD (%)	detected	2.13	0.25	0.0001
	corrected values	2.51	0.53	0.0007

*EMA positive; **According to the data from Szaflarska-Popławska *et al.* (2009)

Table 3. Frequency of HLA-DQ haplotypes predisposing to celiac disease in CF patients and general population

HLA-DQ allele	Number (%)		Statistical significance
	CF	General population	
DQ2	114 (24.8)	113 (28.5)	N.s.
DQ8	44 (9.6)	40 (10.0)	N.s.

tion of CD may be incorrectly related to CF. Mucosal changes in the small intestine and malabsorption related to them may significantly deteriorate the nutritional status and influence potential survival. Therefore, the detection of coexisting diseases may have a significant impact on the effectiveness of treatment in CF patients.

Valletta and Mastella (1989) described five CD cases among 1100 CF patients being treated in a Veronian CF Centre. Therefore, the incidence of CD co-morbidity was calculated to be at least 1:220 (0.45%). In subsequent years (Pardo, 1991), two new cases were detected, suggesting an even higher incidence (approx. 0.6%). However, the risk of CD in the general Italian population was not addressed. A significantly increased gut permeability, probably due to changed mucus characteristics along with increased antigenic load caused by pancreatic insufficiency, was suggested to play a role in favoring gluten-related enteropathy (Cox *et al.*, 1982; Leclecq-Foucart *et al.*, 1987; Murphy *et al.*, 1989). Assessing the frequency of CD in CF patients, Valletta and Mastella (1989) related their data to the incidence of 1:266 (0.38%) in a large group of donors tested for the presence of serum antigliadin antibodies (Hed *et al.*, 1986). Recent data on CD epidemiology points to its quite high incidence in the general population, e.g., 1:99 (1.01%) and 1:106 (0.94%) in Finnish and Italian schoolchildren (Maki *et al.*, 2003; Tommasini *et al.*, 2004), and 1:87 (1.15%) and 1:105 (0.95%) in UK and US adults (Fasano *et al.*, 2003; West *et al.*, 2003). A high prevalence of CD (1.2%) among Scandinavian CF patients was reported (Fluge *et al.*, 2009). The authors referred their data to older publications (at least ten years of difference) showing CD incidence of 1:373 (0.27%), 1:340 (0.29%) and 1:189 (0.53%) in Denmark, Norway and Sweden, respectively (Weile *et al.*, 1996; Hovdenak *et al.*, 1999; Ivarsson *et al.*, 1999). As documented in more recent publications (Maki *et al.*, 2003; West *et al.*, 2003; Lohi *et al.*, 2007), the CD incidence in Nordic countries could be expected to be significantly higher. Therefore, according to the authors' statement, one could argue that the presented co-morbidity just reflect the high prevalence of CD reported lately.

In the present study, two CF subjects were diagnosed as having CD earlier. In six more patients serological screening revealed the presence of EMA. In five of them small bowel biopsy was performed documenting mucosal atrophy in four cases The confirmed (2.13%) and corrected (2.51%) CD incidence in the studied CF population was significantly higher than in the general Polish population, more than eight and almost five-fold, respectively (Table 2). Without any doubt, CF patients with both antibodies positive (tTG and EMA) are at a very high risk of having CD. The only such patient not diagnosed as CD in the present study had a lower tTG titre (54 RU/ml). Four patients with positive tTG and negative EMA were documented to have normal intestinal morphology (Marsh type 0). However, these five CF subjects demand longitudinal follow-up as they might have latent CD being potentially at risk of developing overt CD later.

In contrast to Fluge *et al.*'s study (2009), we compared the results obtained in the present study with recent epidemiological data (Szaflarska-Popławska *et al.*, 2009). In addition, we excluded CF patients younger than three years since serological screening of CD in this age subgroup lacks sensitivity. Although the age of the CF population was diverse, the median value was comparable to the age of the screened schoolchildren. In addition, we assessed the genetic predisposition to CD in the CF population. HLA-DQ2/DQ8 prevalence in the studied and the reference groups was similar (Table 3). The association between the HLA genes (CELIAC1 locus on chromosome 6p21) and CD is very strong as compared to other HLA-linked diseases. However, other factors must be involved (Sollid *et al.*, 2005). It seems that CF is a good example of a strong co-factor promoting CD.

Various explanations of CD co-morbidity with CF could be considered. Intestinal inflammation potentially influencing intestinal permeability (Hendriks *et al.*, 2001) and pancreatic exocrine insufficiency leading to a higher antigen load due to a lack of full digestion (Borowitz *et al.*, 2005) could play a crucial role. Smyth *et al.* (2000) documented increased production of proinflammatory proteins in the gastrointestinal tract of CF patients. Raia *et al.* (2000) found the presence of mononuclear infiltration in lamina propria of duodenal mucosa without any significant changes of its morphology. Increased fecal calprotectin concentrations and rectal NO production were also detected (Bruzzesse *et al.*, 2004). Small intestine bacterial overgrowth (Lisowska *et al.*, 2009) and changes in mucus (Norkina *et al.*, 2004) characteristics may also influence intestinal permeability. Changes in infant-feeding practices have revealed that dietary introduction of gluten while infants are still breast-fed is a protective factor against CD. According to our experience, breast-feeding in CF is rather shorter than in the general population, potentially increasing the risk of CD. Exocrine pancreatic insufficiency involves the contact with higher amounts of non-digested or incompletelly digested dietary products possibly being dietary antigens. In the majority of CF patients exocrine pancreatic insufficiency appears in early infancy (Walkowiak *et al.*, 2005a). Before neonatal CF screening program implementation, the age of diagnosis — at least in a significant fraction of CF patients — was higher than the age of gluten introduction. After implementation of enzyme supplementation its effectiveness is not full (Walkowiak *et al.*, 2005b). Even residual malabsorption in conjunction with high-energy diet still results in a higher antigenic load. Under such circumstances, gluten peptides could more easily cross the epithelium, and after deamidation by tissue transglutaminase be presented by DQ2+ or DQ8+ antigen presenting cells to pathogenic CD4+ cells. T-helper-cell type 1 response may lead to the development of celiac disease (Di Sabatino & Corazza, 2009).

In conclusion, with the obtained data it is tempting to view cystic fibrosis as a risk factor for celiac disease. Our results suggest that cystic fibrosis predisposes to the occurrence of celiac disease with a genetic background similar to that of general population.

CONCLUSIONS

Our results suggest that cystic fibrosis is a risk factor for the occurrence of celiac disease with a genetic predisposition similar to that of general population.

Acknowledgements

Supported by the Ministry of Science and Higher Education (grant No N407 0852 34)

REFERENCES

Bignon JD, Fernandez-Vina MA (1997) Protocols of 12th IHW for typing of HLA class II alleles by DNA amplification by the polymerase chain reaction (PCR) and hybridization with sequence specific oligonucleotide probes (SSOP). In *Genetic diversity of HLA. Functional and medical implication.* Charron D ed, pp 203–204. Paris.

Borowitz D, Durie PR, Clarke LL, Werlin SL, Taylor CJ, Semler J, De Lisle RC, Lewindon P, Lichtman SM, Sinaasappel M, Baker RD, Baker SS, Verkade HJ, Lowe ME, Stallings VA, Janghorbani M, Butler R, Heubi J (2005) Gastrointestinal outcomes and confounders in cystic fibrosis. *J Pediat Gastroenterol Nutr* **41:** 273–285.

Bossuyt X, Blanckaert N (1999) Evaluation of interferences in rate and fixed-time nephelometric assays of specific serum proteins. *Clin Chem* **1:** 62–67.

Bruzzesse E, Raia V., Gaudiello G., Polito G., Buccigrossi V., Fornicola V (2004) Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. *Aliment Pharmacol Ther* **20:** 813–819.

Chorzelski TP, Buetner EH, Sulej J (1984) IgA anti-endomysium antibody. A new immunological marker of dermatitis herpetiformis and celiac disease. *Br J Dermatol* **111:** 395–402.

Cox KL, Isenberg JN, Ament ME (1982) Gastric acid hypersecrection in cystic fibrosis. *J Pediatr Gastroenterol Nutr* **1:** 559–565.

Di Sabatino A, Corazza GR (2009) Coeliac disease. *Lancet* **373:** 1480–1493.

Dietrich W, Ehinis T, Bauer M (1997) Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* **3**: 797–801.

Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath 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* **163:** 286–292.

Fluge G, Olesen HV, Gilljam M, Meyer P, Pressler T, Storrösten OT (2009) Co-morbidity of cystic fibrosis and celiac disease in Scandinavian cystic fibrosis patients. *J Cyst Fibros* **8:** 198–202.

Hed J, Lieden G, Ottosson E, Ström M, Walan A, Groth O, Sjögren F, Franzen L (1986) IgA antigliadin antibodies and jejunal mucosal lesions in healthy blood donors. *Lancet* **11:** 215.

Hendriks HJ, van Kreel B, Forget PP (2001) Effects of therapy with lansoprazole on intestinal permeability and inflammation in young cystic fibrosis patients. *J Pediatr Gastroenterol Nutr* **33:** 260–265.

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

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

Leclercq-Foucart J, Forget P, Van Cutsem JL (1987) Lactulose rhamnose intestinal permeability in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr* **6:** 66–70.

Lisowska A, Wójtowicz J, Walkowiak J (2009) Small intestine bacterial overgrowth is frequent in cystic fibrosis: combined hydrogen and methane measurements are required for its detection. *Acta Biochim Pol* **56:** 631–634.

Lohi S, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, Lohi O, Bravi E, Gasparin M, Reunanen A, Mäki M (2007) Increasing prevalence of celiac disease over time. *Aliment Pharmacol Ther* **26:** 1217–1225.

Maki M, Mustalahiti K, Kokkonen J, Kulmala P, Haapalahti M, Karttunen T, Ilonen J, Laurila K, Dahlbom I, Hansson T, Höpfl P, Knip M (2003) Prevalence of celiac disease among children in Finland. *N Engl J Med* **348:** 2517–2524.

Marsh MN (1992) Gluten, major histocompatibility complex, and the small intestine. A molecular and immunologic approach to the spectrum of gluten sensitivity (celiac spurue). *Gastroenterology* **102:** 330–354.

Murphy MS, Sheldon W, Brunetto A, Pearson AD, Laker MF, Eastham EJ, Nelson R (1989) Active and passive sugar absorption in pancreatic insufficiency. *J Pediatr Gastroenterol Nutr* **8:** 189–194.

Norkina O, Kaur S, Ziemer D, De Lisle RC (2004) Inflammation of the cystic fibrosis mouse small intestine. *Am J Physiol Gastrointest Liver Physiol* **286:** 1032–1041.

Oberhuber G, Granditsch G, Vogelsang H (1999) The histopatopathology of celiac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* **11:** 1185–1194.

Pardo F (1991) Coesistenza di malattia celiaca e di fibrosi cistica. Descrizione di un caso clinico. *Riv Ital Pediatr* **17:** 614.

Raia V, Maiuri L, De Ritis G, de Vizia B, Vacca L, Coute R (2000) Evidence of chonic inflammation in morphologically normal small intestine of cystic fibrosis patients. *Pediatr Res* **47:** 344–350.

Smyth RL, Croft NM, O'Hea U, Marshall TG, Ferguson A (2000) Intestinal inflammation in cystic fibrosis. *Arch Dis Child* **82:** 394–399.

Sollid LM, Lie BA (2005) Celiac disease genetics: current concepts and practical applications. *Clin Gastroenterol Hepatol* **3:** 843–851.

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

Szaflarska-Popławska A, Parzęcka M, Müller L, Placek W (2009) Screening for celiac disease in Poland. *Med Sci Monit* **15:** PH7–PH11.

Tommasini A, Not T, Kiren V, Baldas V, Santon D, Trevisiol C, Berti I, Neri E, Gerarduzzi T, Bruno I, Lenhardt A, Zamuner E, Spanò A, Crovella S, Martellossi S, Torre G, Sblattero D, Marzari R, Bradbury A, Tamburlini G, Ventura A (2004) Mass screening for coeliac disease using antihuman transglutaminase antibody assay. *Arch Dis Child* **89:** 512–515.

Valleta EA, Mastella G (1989) Incidence of celiac disease in a cystic fibrosis population. *Acta Paediatr Scand* **78:** 784–785.

Venuta A, Bertolani P (1999) Coexistence of cystic fibrosis and celiac disease. Description of a clinical case and reviev of the literature. *Pediatr Med Chir* **21:** 223–226.

Walkowiak J, Nousia-Arvanitakis S, Henker J, Stern M, Sinaasappel M, Dodge JA (2005) Indirect pancreatic tests in children. *J Pediatr Gastroenterol Nutr* **40:** 107–114.

Walkowiak J, Sands D, Nowakowska A, Piotrowski R, Zybert K, Herzig KH, Milanowski A (2005) Early decline of pancreatic function in cystic fibrosis patients with lass 1 or 2 CFTR mutations. *J Pediatr Gastroenterol Nutr* **40:** 199–201.

Weile B, Godzinsky E, Skogh T, Jordal R, Cavell B, Krasilnikoff PA (1996) Screening Danish blood donors for antigliadin and antiendomysium antibodies. *Acta Pediatr (Suppl)* **412:** 46.

West J, Logan RF, Hill PG, Lloyd A, Lewis S, Hubbard R, Reader R, Holmes GK, Khaw KT (2003) Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. *Gut* **52:** 960–965.