http://www.medscape.com/viewarticle/573548

Advances in Coeliac Disease

John M Hutchinson; Gerry Robins; Peter D Howdle
Curr Opin Gastroenterol. 2008;24(2):129-134.
Posted 05/21/2008

Abstract and Introduction

Abstract

Purpose of review: The number of people diagnosed with coeliac disease continues to rise, and this article critically summarizes recent research into the condition.
Recent findings: Much work has been focused on clarifying the molecular pathways involving cytokines in coeliac disease. Such work will yield improved understanding of the complex pathogenesis of coeliac disease and novel therapeutic targets.
Summary: The recent literature predominantly focuses on both elucidating the pathogenesis and improving diagnostic strategies for coeliac disease, but further work into the treatment of coeliac disease is needed.

Introduction

This article reviews important publications in the field of coeliac disease published between January 2006 and July 2007. It concentrates on developments in screening, diagnosis, pathogenesis and therapy.

Screening

Coeliac disease is common but it can be difficult to detect based on clinical symptoms alone. Early intervention can modify disease progress, and this makes coeliac disease a good candidate for screening. Screening can be targeted (i.e. aimed at high-risk groups) or nontargeted (population screening). Since 1997 serological [endomysial antibody (EMA) and anti-tissue transglutaminase (anti-tTG)] screening has been offered to all first-degree and second-degree relatives of patients with coeliac disease in south east Wales.[1] Patients diagnosed with coeliac disease by relative screening (n = 32) were younger than those diagnosed routinely (median age 33 years versus 54 years) and suffered less from osteoporosis (9% versus 22%), anaemia (13% versus 58%) and other complications at diagnosis. This may reflect the fact that more were diagnosed at an earlier stage of coeliac disease development. In a US-based screening of 171 family members (88% were first-degree relatives and 12% were second-degree relatives) who had previously had a negative EMA,[2] six subsequently seroconverted.[3] The mean time to seroconversion was 1.7 years (range 0.5-3.17 years). One-time screening may therefore be insufficient to detect all those who will develop coeliac disease, although this was a high-risk group.

Another study of first-degree relatives was conducted to evaluate the usefulness of human leucocyte antigen (HLA)-DQ2 genotyping.[4] HLA serology in 221 first-degree relatives of 82 DQ2-positive coeliac disease patients was assessed, and duodenal biopsies for histological examination were taken in those who were DQ2 positive. Biochemical parameters and bone mineral density were recorded. A total of 130 relatives (58.8%) were DQ2 positive, with 64 of these (49.2%) having Marsh 0 (i.e. 'normal') lesions, 32 (24.6%) Marsh I [i.e. increased intraepithelial lymphocytes (IELs) only], one (0.8%) Marsh II, and 13 (10.0%) Marsh III; 20 individuals (15.4%) refused to undergo biopsy. Relatives with Marsh I lesions were more often symptomatic (56.3%) and anaemic (21.4%) than were those with Marsh 0 lesions (21.1% and 6.2%, respectively). The prevalence of abnormal bone mineral density was similar between those relatives with Marsh I lesions (37%) and Marsh III lesions (44.4%). The authors concluded that villous atrophy is not necessary to recommend a gluten-free diet (GFD), at least in DQ2-positive relatives of coeliac disease patients. Bourgey *et al.*[5] evaluated the HLA-related genetic risk that future siblings of children with coeliac disease will also develop coeliac disease. In a cohort of 188 Italian families, in which at least one sibling and both parents were tested, the overall risk for a sibling to develop coeliac disease was approximately 10% (range 0.1-29%).

A clinical decision tool that uses pre-endoscopy serological testing and assessment of symptoms in patients attending for gastroscopy was devised and assessed by Hopper *et al.*[6] An initial retrospective cohort of 1464 unselected

patients who underwent duodenal biopsy was used to design the tool. The prevalence of coeliac disease cases was 4.2% in this group. Based on these the new clinical decision tool required all patients to have their anti-tTG tested in primary care (along with their IgA status), allowing stratification into three categories: those with high-risk symptoms such as diarrhoea, weight loss and anaemia, who would undergo duodenal biopsy regardless of the anti-tTG result; those with low-risk symptoms but who were anti-tTG positive, who would also have had a duodenal biopsy; and those with low-risk symptoms and negative anti-tTG, in whom a duodenal biopsy would not be performed. This tool was tested prospectively on a second cohort of 2000 patients, who again all underwent duodenal biopsy. If the clinical decision tool had been used, 58.5% of the patients would have avoided a duodenal biopsy while identifying the same number of patients with coeliac disease.

Shamir *et al.*[7] considered a nontargeted group of 1571 healthy blood donors and how screening for coeliac disease affected patients' lifestyles and attitudes. The authors had initially found that 3.8% of individuals had positive serology suggestive of coeliac disease [EMA or gliadin antibody (AGA) or tTG]. Of the 10 patients diagnosed with coeliac disease (based on serology and biopsy) only four adhered to a GFD, and only one had his family members screened. Of the 17 individuals diagnosed as possibly having coeliac disease on the basis of serology alone (i.e. normal mucosa), only one took up the offer of repeated serology.

Diagnosis

The 'gold standard' for diagnosis remains histology, although for screening purposes serology is used.

Serology

The best way to test for coeliac disease serologically remains controversial, with no defined standard regarding the use of EMA, tTG and AGA. Reeves *et al.*[8] used a multicentre approach to define the optimal screening for coeliac disease, considering whether IgA-tTG ± IgG-tTG could be used as a replacement for EMA. Dual-isotype (i.e. use of more than one type of kit) transglutaminase (tTG-dual), combined-isotype transglutaminase (IgA-tTG + IgG-tTG), IgA-tTG, combined-isotype AGA (IgA-AGA + IgG-AGA), IgA-AGA, and EMA assays were compared. The cohort included 254 patients but 228 were 'biopsy negative', which calls into question whether these patients did indeed have coeliac disease. The protocol tested for IgA-tTG, followed by IgG-tTG if negative. IgG-tTG increased diagnostic sensitivity for coeliac disease in IgA-deficient individuals with a sensitivity of 100% for dual-isotype tTG versus 66.67% for IgA-tTG. For the 10.6% of individuals with reduced IgA levels, the dual or combined isotype-tTG testing approach was superior to either isotype measurement in isolation. Total IgA measurement did not provide enhanced diagnostic sensitivity when dual-isotype tTG testing was employed. Serological assessment of coeliac disease with tTG screening (IgA ± IgG isotype) was concluded to be an alternative to EMA. This study also confirms that AGA antibody testing no longer appears to be a part of the diagnostic strategy.

Sinclair *et al.*[9] sought an easier and more cost-effective way to exclude IgA deficiency without measuring total serum IgA. Optical density readings on enzyme-linked immunosorbent assays of 608 routine samples received for tTG antibody testing for coeliac disease were compared with total IgA concentrations. A linear correlation between optical density values and levels of serum IgA was seen, such that the latter only needed to be tested for its absence if the optical density was under 0.050 on enzyme-linked immunosorbent assay. Then, if total IgA is within the age-related reference range, the negative tTG antibody can be reported.

Another possible use of serological markers is to predict severity of mucosal damage. IgA antiactin antibodies (IgA-AAA) are circulating autoantibodies directed toward the intracellular cytoskeleton actin filaments.[10,11] Carroccio *et al.*[12] reported a four centre study conducted to evaluate their potential role in monitoring intestinal mucosal lesions. The study included 205 patients with newly diagnosed coeliac disease with villous atrophy, 80 healthy control individuals and 81 disease control individuals. Serum IgA-AAA values were significantly higher in patients with coeliac disease than in healthy or disease control individuals ($P < 0.0001$). The serum levels were positive in 41 of the 60 coeliac disease patients with mild intestinal histological lesions (69%) and in 123 of the 145 with severe lesions (85.3%; $P < 0.05$). Serum IgA-AAA expression correlated ($P < 0.0001$) with the severity of intestinal damage, even in those on a self-reported GFD.

Levels of soluble CD163, a scavenger receptor[13] shed by tissue macrophages, have been shown to be higher in patients with untreated coeliac disease than in those with treated disease and to correlate with severity of the coeliac lesion, as classified according to the Marsh criteria.[14] Similarly, expression of matrix metalloproteinases (MMPs), which belong to a family of neutral proteases that are capable of degrading extracellular matrix and basement

membrane components, were recently examined in duodenal biopsies of 30 patients with coeliac disease.[15] There was increased expression of this family of proteases in coeliac disease and high correlation with mucosal damage. The authors hypothesized that MMPs may have an additional, as yet unidentified role within the innate immune system and development of coeliac disease, as well as its usual degradation role, supporting earlier work.[16,17]

Histology

Difficulty often arises when a patient who is suspected of having coeliac disease has a negative EMA result with a borderline histology. In 883 patients who underwent upper gastrointestinal endoscopy at Tampere University Hospital, Finland between 1995 and 2000 and in whom small bowel biopsy was performed when coeliac disease was suspected, regardless of antibody result, villous atrophy and crypt hyperplasia were found in 177 (21%) patients.[18] The clinical and histological features of IgA-competent, EMA-negative coeliac disease patients were compared with those in EMA-positive patients; the investigators also determined whether tTG-specific IgA deposits could be found in small bowel mucosa, even in the patients with seronegative tTG coeliac disease. Of those with coeliac disease, 26 (15%) had negative serum EMA, of which four were IgA deficient. The presence or otherwise of tTG-specific IgA deposits in the small bowel mucosa could help in cases with ambiguous histology, but the lack of a gold standard in these cases makes interpretation difficult.

Pathogenesis

Numerous factors are important in the development of coeliac disease, and these are discussed below.

Human Leucocyte Antigen Factors

Jores *et al.*[19] identified a clear-cut correlation between those homozygous for the HLA-DQB1*0201 allele and the extent of intestinal damage, but not the clinical presentation.

Non-human Leucocyte Antigen Factors

Rho-GTPase proteins such as myosin IXB (encoded by *MY09B*) are known to play a role in epithelial cytoskeletal organization. *MYO9B* polymorphisms have been identified as a predisposing factor for coeliac disease in analyses conducted by different European groups.[20-22] Recent data suggest that variation in *MYO9B* gene polymorphisms does not appear to have a major effect on coeliac disease in the UK[23] and South Italian[24] populations. Other genetic associations recently considered include the *SPINK* (serine protease inhibitors of the Kazal type) genes, which play a role in tissue preservation through the containment of uncontrolled proteolysis and bacterial growth. In the Dutch population evaluated by Wapenaar *et al.*[25] no risk linkage was identified, in contrast to previous work.

In a large multicentre study, van Heel *et al.*[26] conducted a genome-wide association study for coeliac disease and identified risk variants in the interleukin (IL)-2/IL-21 region of chromosome 4.

Cytokines

Aberrant T cell populations play an essential role in the pathogenesis of coeliac disease and refractory coeliac disease/enteropathy associated T-cell lymphoma. De Re *et al.,*[27] using two-dimensional difference gel electrophoresis, investigated the proteins associated with an aberrant T-cell population in refractory coeliac disease. Significantly higher levels of IgM, apolipoprotein C-III (which is an inhibitor of lipoprotein lipase) and Charcot-Leyden crystal proteins (eosinophil-specific granule protein) were demonstrated in a duodenal biopsy specimen of the patient with refractory coeliac disease compared with biopsies from four patients with coeliac disease.

The pathology of coeliac disease is associated with an expansion of IELs, both _ß and __, in the damaged mucosa. Kolkowski *et al.*[28] presented a report on the cytokine profile of $CD8^+$ _ß IELs as compared with clones derived from noncoeliac disease donors. An imbalance in the production of IL-10 and IL-2 was observed. Coeliac disease clones capable of high toxicity produced IL-2, whereas most cytotoxic noncoeliac disease IELs produced IL-10. This finding may also be significant, given the low generation of regulatory $CD8^+$ IELs (that produce IL-10) seen in coeliac disease. Such work suggests that the imbalance between functionally distinct IEL populations resident in the small bowel may be involved in the pathogenesis of coeliac disease, primarily through the decrease in IL-10 producing IELs.

It has become increasingly recognized that natural killer cells have an important role to play as immunoregulatory cells in the pathogenesis of a number of autoimmune conditions such as type I diabetes mellitus[29] and systemic sclerosis.[30] Grose *et al.*[31] investigated whether a deficiency in number and function of invariant natural killer cells (which produce IL-4 and interferon-_, and hence suppress the T-helper-1 response) was present in coeliac disease. They found that patients with coeliac disease were deficient in invariant natural killer cells, which were functionally defective in producing IL-4. This could allow a T-helper-1 rather than a T-helper-2 immune response to contribute to the inappropriate activation of gluten-sensitized T cells.

A tumour necrosis factor-_-308 polymorphism ($^{-308}$A) variant, which consists of a guanine to adenine transition, has been associated with enhanced tumour necrosis factor-_ production. Data were collected on the frequency of this polymorphism in children with coeliac disease and type I diabetes mellitus, and of a similar transition at position 238 ($^{-238}$A), although the exact effect of this on tumour necrosis factor-_ production (if any) is as yet unknown.[32] $^{-238}$A is known to be associated with increased severity of several autoimmune diseases. There was no evidence that the $^{-308}$A carrier state conferred additional risk for coeliac disease in type I diabetes mellitus. There was, however, a significantly higher rate of $^{-238}$A in the histology-proven coeliac disease group than in a noncoeliac disease group with type I diabetes mellitus.

There is growing evidence for a role in coeliac disease pathogenesis for IL-15,[33,34] which has multiple functions at the interface between innate and adaptive immunity. Benahmed *et al.*[35] demonstrated that IL-15 is markedly over-expressed in the mucosa of patients with active coeliac disease. This observation is consistent with the finding that transforming growth factor-ß signalling in mucosal T cells is impaired by the heightened expression of IL-15, leading to sustained intestinal inflammation and injury.[36]

Another cytokine that has raised interest is macrophage migration inhibiting factor, a peptide that is expressed by various cells, such as monocytes/macrophages and eosinophils. Nunez *et al.*[37] studied the frequency, in 531 Spanish patients with coeliac disease, of their chosen migration inhibiting factor susceptibility marker (a $[CAAR]_{5-8}$ tetranucleotide repeat at position -794), which has strong linkage disequilibrium with a transition at position -173 of guanine to cytosine. Their work suggested that this haplotype, which is associated with several other autoimmune diseases, significantly increases the risk for coeliac disease (odds ratio = 1.32).

Other Predisposing Candidates for Coeliac Disease

The intercellular adhesion molecule-1 gene is a good candidate for coeliac disease predisposition because its encoded protein acts as an adhesion and co-stimulatory receptor for transendothelial migration of neutrophils to inflammatory sites. Abel *et al.*[38] investigated the contribution of intercellular adhesion molecule-1 to coeliac disease risk by analysing the frequency of two frequent single nucleotide polymorphisms. One single nucleotide polymorphism, resulting in a change at position 241 of glycine to arginine, predisposed to adulthood-onset coeliac disease in French Caucasian patients (odds ratio = 4.2). Unfortunately, the functional consequences of this frequent polymorphism are not known.

Treatment

GFD remains the only available therapy for coeliac disease. Alternatives are being actively investigated.

Gluten-free Diet

Gluten-free products are not always widely available and are usually more expensive than their gluten-containing counterparts. This problem has been highlighted again,[39] as has the diversity in gluten product consumption and labelling standards across the European Union (currently, this can vary from 20 to 200 parts per million). Based on the need to establish a safe pan-European Union threshold, Catassi *et al.*[40] aimed to establish the safety threshold of prolonged exposure to trace amounts of contaminating gluten. They conducted a multicentre (albeit all Italian), double-blind, placebo-controlled randomized trial in 49 adults with biopsy-proven coeliac disease who were on a GFD. Abnormal intestinal morphology persisted in a significant proportion of coeliac disease patients being treated with a GFD, possibly due to the persisting ingestion of hidden gluten. An intake of 50 mg gluten per day produced significant mucosal damage, suggesting that gluten should be kept to lower levels than this when treating coeliac disease.

An alternative treatment strategy is to 'neutralise' gluten once it has been ingested. A decapeptide derived from durum wheat (10mer sequence QQPQDAVQPF) was recently shown to inhibit the lymphocyte response to gliadin peptides,[41]

suggesting that potentially new therapeutic approaches to coeliac disease may be found in naturally occurring toxic cereals.

Enzymatic Degradation

In the search for new treatments to help reduce the impact that following a GFD has on a patient's lifestyle, two recent studies from the same group in Amsterdam[42,43] were conducted to determine the efficiency of gluten degradation by a postproline cutting enzyme, namely prolyl endoprotease from *Aspergillus niger.* The authors showed that prolyl endoprotease from *A. niger* can act under conditions similar to those found in the gastrointestinal tract and is capable of degrading intact gluten molecules and T-cell-stimulatory epitopes from gluten into harmless fractions. Work by Garcia-Horsman *et al.,*[44] however, appeared to rule out any significant role in coeliac disease, because they found that prolyl endoprotease was unable to eliminate the gliadin-derived immunoactive and toxic peptides larger than 33mer. They did comment that because of the lack of an animal model, the in-vivo efficacy would have to be ultimately addressed in clinical studies involving coeliac disease patients.

Conclusion

Although much research is still focused, quite rightly, on pathogenesis of and susceptibility to coeliac disease, little progress appears to have been made in the treatment of coeliac disease over and above the use of a GFD, but even this strategy is compromised by differing international standards. More research into the use of biotechnologies that decrease the risk for gluten contamination in both 'gluten free' products and 'normal' foods is desirable. In the interim, however, efforts to improve the accuracy of coeliac disease diagnosis must continue, both to inform those with unknown coeliac disease of the potential consequences and to limit the number of patients who may be diagnosed with this condition in error.

References

1. Sundar N, Crimmins R, Swift G. Clinical presentation and incidence of complications in patients with coeliac disease diagnosed by relative screening. Postgrad Med J 2007; 83:273-276. This study compared clinical features in coeliac disease patients detected via routine testing with those diagnosed through family screening. It highlights the potential advantage of utilizing relative screening.
2. Fasano A, Berti I, Gerarduzzi T, *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; 163:286-292.
3. Goldberg D, Kryszak D, Fasano A, Green PH. Screening for celiac disease in family members: is follow-up testing necessary? Dig Dis Sci 2007; 52:1082-1086. Using a database maintained at the University of Maryland Center for Celiac Research, USA, the authors aimed to assess whether one-time screening of family members is sufficient and to establish the time interval for repeat testing in those family members who initially tested negative. The conclusion was that one-time testing in individuals is insufficient.
4. Esteve M, Rosinach M, Fernandez-Banares F, *et al.* Spectrum of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease: clinical relevance of lymphocytic enteritis. Gut 2006; 55:1739-1745. This study included 221 first-degree relatives of 82 DQ2-positive patients with coeliac disease. The authors showed that a high number of patients with Marsh 1 lesions were symptomatic for abdominal distension and pain. In summary, the study shows the potential benefit a GFD would have for such patients, and it substantiates the need for other diagnostic strategies to improve coeliac disease detection rates and the potential role that HLA-DQ2 genotyping may play in this matter.
5. Bourgey M, Calcagno G, Tinto N, *et al.* HLA related genetic risk for coeliac disease. Gut 2007; 56:1054-1059. This study of a cohort of 188 Italian families was conducted to evaluate the risk that siblings of children with coeliac disease will also develop coeliac disease and to genotype all family members. Such studies provide direction for calculating coeliac disease risk, thus potentially tailoring screening to an individual's risk in the antenatal setting.
6. Hopper AD, Cross SS, Hurlstone DP, *et al.* Preendoscopy serological testing for coeliac disease: evaluation of a clinical decision tool. BMJ 2007; 334:729-733. Using a retrospective analysis the authors analysed data from 1464 unselected patients who had undergone duodenal biopsy. Their aim was to develop an effective diagnosis tool for detecting all cases of coeliac disease in patients referred for gastroscopy without performing duodenal biopsy. Alongside cost-effectiveness, the study has implications for avoiding the need to do duodenal biopsies in low-risk patients.
7. Shamir R, Yehezkely-Schildkraut V, Hartman C, Eliakim R. Population screening for celiac disease: follow up of patients identified by positive serology. J Gastroenterol Hepatol 2007; 22:532-535. The authors offered endoscopy with intestinal biopsy to the 59 patients with positive serology from their original study on the prevalence of coeliac disease in healthy blood donors in Israel. They found that investigation and management of coeliac disease still has obstacles to overcome if coeliac disease screening is to provide enhanced health benefits.
8. Reeves GE, Squance ML, Duggan AE, *et al.* Diagnostic accuracy of coeliac serological tests: a prospective study. Eur J Gastroenterol Hepatol 2006; 18:493-501. A prospective multicentre serology study was performed throughout Australia. It was found that dual-isotype tTG is a sensitive and specific alternative to EMA testing in the serological assessment of

coeliac disease.
9. Sinclair D, Saas M, Turk A, *et al.* Do we need to measure total serum IgA to exclude IgA deficiency in coeliac disease? J Clin Pathol 2006; 59:736-739. The authors at the Department of Clinical Biochemistry at the Queen Alexandra Hospital, Portsmouth, UK utilized optical density readings on enzyme-linked immunosorbent assay of 608 routine samples received for tTG testing for coeliac disease and compared them with total IgA concentrations. In brief, they identified an easier and more cost-effective and practical way to exclude IgA deficiency in coeliac disease.
10. Clemente MG, Musu MP, Frau F, *et al.* Immune reaction against the cytoskeleton in coeliac disease. Gut 2000; 47:520-526.
11. Clemente MG, Musu MP, Troncone R, *et al.* Enterocyte actin autoantibody detection: a new diagnostic tool in celiac disease diagnosis: results of a multicenter study. Am J Gastroenterol 2004; 99:1551-1556.
12. Carroccio A, Brusca I, Iacono G, *et al.* IgA antiactin antibodies ELISA in coeliac disease: a multicentre study. Dig Liver Dis 2007; 39:818-823. In this study four centres in Italy recruited 205 newly diagnosed coeliac disease patients with villous atrophy between January and December 2004. The authors aimed to evaluate the clinical usefulness of serum IgA-AAA in monitoring intestinal mucosal lesions while taking a GFD. They concluded that using simple ELISA IgA-AAA is a reliable marker of severe intestinal damage.
13. Kristiansen M, Graversen JH, Jacobsen C, *et al.* Identification of the haemoglobin scavenger receptor. Nature 2001; 409:198-201.
14. Daly A, Walsh C, Feighery C, *et al.* Serum levels of soluble CD163 correlate with the inflammatory process in coeliac disease. Aliment Pharmacol Ther 2006; 24:553-559. The authors from St James's Hospital Dublin, Ireland aimed to determine whether levels of soluble CD163 correlated with the inflammatory lesion in coeliac disease. Its use in evaluating the effectiveness of the diet in reducing the inflammatory lesion may have a prominent role in the future.
15. Mohamed BM, Feighery C, Kelly J, *et al.* Increased protein expression of matrix metalloproteinases -1, -3, and -9 and TIMP-1 in patients with gluten-sensitive enteropathy. Dig Dis Sci 2006; 51:1862-1868.
16. Daum S, Bauer U, Foss HD, *et al.* Increased expression of mRNA for matrix metalloproteinases-1 and -3 and tissue inhibitor of metalloproteinases-1 in intestinal biopsy specimens from patients with coeliac disease. Gut 1999; 44:17-25.
17. Ciccocioppo R, Di SA, Bauer M, *et al.* Matrix metalloproteinase pattern in celiac duodenal mucosa. Lab Invest 2005; 85:397-407.
18. Salmi TT, Collin P, Korponay-Szabo IR, *et al.* Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut 2006; 55:1746-1753.
19. Jores RD, Frau F, Cucca F, *et al.* HLA-DQB1*0201 homozygosis predisposes to severe intestinal damage in celiac disease. Scand J Gastroenterol 2007; 42:48-53.
20. van Belzen MJ, Meijer JW, Sandkuijl LA, *et al.* A major non-HLA locus in celiac disease maps to chromosome 19. Gastroenterology 2003; 125:1032-1041.
21. Babron MC, Nilsson S, Adamovic S, *et al.* Meta and pooled analysis of European coeliac disease data. Eur J Hum Genet 2003; 11:828-834.
22. Monsuur AJ, de Bakker PI, Alizadeh BZ, *et al.* Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. Nat Genet 2005; 37:1341-1344.
23. Hunt KA, Monsuur AJ, McArdle WL, *et al.* Lack of association of MYO9B genetic variants with coeliac disease in a British cohort. Gut 2006; 55:969-972.
24. Cirillo G, Di Domenico MR, Corsi I, *et al.* Do MYO9B genetic variants predispose to coeliac disease? An association study in a cohort of South Italian children. Dig Liver Dis 2007; 39:228-231. Further to previous work, the authors aimed to verify the effects of the *MYO9B* polymorphism on disease risk in a Mediterranean population of children with coeliac disease. Their results were in accordance with the UK study.[23]
25. Wapenaar MC, Monsuur AJ, Poell J, *et al.* The SPINK gene family and celiac disease susceptibility. Immunogenetics 2007; 59:349-357. The Dutch authors aimed to assess the gut mucosal gene expression and genetic association of *SPINK* 1, 2, 4 and 5 in the Dutch coeliac disease population. The authors of this initial work were unable to find an association with coeliac disease.
26. van Heel DA, Franke L, Hunt KA, *et al.* A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nat Genet 2007; 39:827-829.
27. De Re V, Simula MP, Caggiari L, *et al.* Proteins specifically hyperexpressed in a coeliac disease patient with aberrant T cells. Clin Exp Immunol 2007; 148:402-409. These Spain-based authors set out to compare the cytokine profile and cytoxicity pattern from $CD8^+$ IEL clones isolated from coeliac disease and noncoeliac disease biopsies. In summary, they found that the imbalance between functionally distinct IEL populations that reside in the small intestinal mucosa may play a role in the pathogenesis of coeliac disease.
28. Kolkowski EC, Fernandez MA, Pujol-Borrell R, Jaraquemada D. Human intestinal alphabeta IEL clones in celiac disease show reduced IL-10 synthesis and enhanced IL-2 production. Cell Immunol 2006; 244:1-9.
29. Baxter AG, Kinder SJ, Hammond KJ, *et al.* Association between alphabetaTCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. Diabetes 1997; 46:572-582.
30. Sumida T, Sakamoto A, Murata H, *et al.* Selective reduction of T cells bearing invariant V alpha 24J alpha Q antigen receptor in patients with systemic sclerosis. J Exp Med 1995; 182:1163-1168.
31. Grose RH, Cummins AG, Thompson FM. Deficiency of invariant natural killer T cells in coeliac disease. Gut 2007; 56:790-795.
32. Hermann C, Krikovszky D, Vasarhelyi B, *et al.* Polymorphisms of the TNF-alpha gene and risk of celiac disease in T1DM children. Pediatr Diabetes 2007; 8:138-141.
33. Mention JJ, Ben AM, Begue B, *et al.* Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology 2003; 125:730-745.
34. Maiuri L, Ciacci C, Ricciardelli I, *et al.* Association between innate response to gliadin and activation of pathogenic T cells

in coeliac disease. Lancet 2003; 362:30-37.
35. Benahmed M, Meresse B, Arnulf B, *et al.* Inhibition of TGF-beta signaling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease. Gastroenterology 2007; 132:994-1008. This French study was conducted to investigate why the proinflammatory effects of IL-15 cannot efficiently be controlled by transforming growth factor-ß in coeliac disease. Their study supplies evidence of the potential role of IL-15 in promoting and sustaining intestinal inflammation in coeliac disease through impairment of transforming growth factor-ß signalling.
36. Kagnoff MF. Mucosal inflammation in celiac disease: interleukin-15 meets transforming growth factor beta-1. Gastroenterology 2007; 132:1174-1176. This compact report collates and summarizes the collecting body of research showing the pivotal role IL-15 plays in impeding transforming growth factor-ß signalling in mucosal T-cells and hence its functional relevance in coeliac disease pathogenesis.
37. Nunez C, Rueda B, Martinez A, *et al.* Involvement of macrophage migration inhibitory factor gene in celiac disease susceptibility. Genes Immun 2007; 8:168-170. This short communication from these Spanish authors describes their testing of the influence of two functional *MIF* promotor variants in coeliac disease susceptibility. Their work produces early evidence for the role of the *MIF* gene in coeliac disease development.
38. Abel M, Cellier C, Kumar N, *et al.* Adulthood-onset celiac disease is associated with intercellular adhesion molecule-1 (ICAM-1) gene polymorphism. Hum Immunol 2006; 67:612-617. The authors studied 180 unrelated French Caucasian patients. Their results indicate that intercellular adhesion molecule genetic variants may play a role in coeliac disease pathogenesis, but it is a small study.
39. Gibert A, Espadaler M, Angel Canela M, *et al.* Consumption of gluten-free products: should the threshold value for trace amounts of gluten be at 20, 100 or 200 p. p. m.? Eur J Gastroenterol Hepatol 2006; 18:1187-1195.
40. Catassi C, Fabiani E, Iacono G, *et al.* A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr 2007; 85:160-166. The objective in this study was to establish the safe threshold of prolonged exposure to trace amounts of gluten (i.e. contaminating gluten) using a multicenter, double-blind, placebo-controlled randomized trial in 49 adults with biopsy-proven coeliac disease who were being treated with a GFD for over 2 years. The investigators found that abnormal bowel morphology persisted in a significant portion of patients on a GFD.
41. Silano M, Leonardi F, Trecca A, *et al.* Prevention by a decapeptide from durum wheat of in vitro gliadin peptide-induced apoptosis in small-bowel mucosa from coeliac patients. Scand J Gastroenterol 2007; 42:786-787. Building on the theory that by identifying an antagonist peptide, which can inhibit the abnormal immune response triggered by gliadin peptides in coeliac disease, the authors assessed the ability of a 10mer decapeptide from the alcohol-soluble protein fraction of durum wheat to do just that.
42. Mitea C, Havenaar R, Drijfhout JW, *et al.* Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for celiac disease. Gut 2007; 57:25-32. These Dutch authors aimed to determine the efficiency of gluten degradation by prolyl endoprotease from *Apergillus niger* in what they describe as a dynamic system that closely mimics the human gastrointestinal tract. Their work implies that co-administration of *A. niger* prolyl endoprotease with a gluten containing meal might potentially eliminate gluten toxicity.
43. Stepniak D, Spaenij-Dekking L, Mitea C, *et al.* Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. Am J Physiol Gastrointest Liver Physiol 2006; 291:G621-G629.
44. Garcia-Horsman JA, Venalainen JI, Lohi O, *et al.* Deficient activity of mammalian prolyl oligopeptidase on the immunoactive peptide digestion in coeliac disease. Scand J Gastroenterol 2007; 42:562-571.

Reprint Address

Professor Peter Howdle, Academic Medical Unit, St James's University Hospital, Leeds LS97TF, UK Tel: +44 113 206 5256; fax: +44 113 242 9722. E-mail: p.d.howdle@leeds.ac.uk .

John M Hutchinson, **Gerry Robins**, **Peter D Howdle**, Academic Medical Unit, Leeds Institute of Molecular Medicine, Leeds, UK