

Coeliac Disease

With processed-grain products a staple of developed nations, is gluten intolerance becoming the under-diagnosed epidemic of the 21st century, asks Dr. Carole Hungerford.

Carole Hungerford, BA, MBBS, FACNEM, is practicing GP

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Summary

- Diagnosing Coeliac Disease can be clinically rewarding: some patients may be surprised that they will have a chronic illness. Asymptomatic patients usually identify with implications for families
- One in a hundred Australians are celiac but only one in 10 of those are diagnosed. The rest suffer various degrees of illness that is treatable by diet.
- A diagnosis of celiac disease may also help identify or prevent associated conditions such as small-intestinal adenocarcinoma, infertility, enteropathy-associated T-cell lymphoma, dementia, thyroiditis and diabetes type 1. As this extends across families, a celiac screen by serology may be highly cost effective.
- Coeliac screening is an ideal test. It is cheap, low invasive and reliable, and an effective treatment exists.

Syphilis has always been referred to as the great mimic. Lupus is similarly the 'wolf in sheep's clothing'. Indeed, lupus has often been called the archetypal auto-immune disease. However, in 2007, coeliac disease (CD) could rightly wear both these crowns of mimicry and auto-immunity. It has long been my contention that one could probably teach a large part of the medical degree by studying this one disease. It is at once an auto-immune disease, a genetic disorder and a nutritional-deficiency disease, showing how these factors can interact. It runs the gamut from neurology through to malignant disease.

Why, then, is it one of the least looked for and understood of medical conditions? It could be that we are trained to think in terms of symptoms more than process. The cynic might remark that there is no drug that can cure coeliac. Without drug-company promotion, will it continue to be something of a Cinderella? Or is it because it is often treated by naturopaths that doctors have trouble taking it seriously? Let us look at it another way.

We would not monitor a pregnancy without screening for rubella. For years, the syphilis screen has been *de rigueur*: However, undiagnosed CD in pregnancy is 1200 times more likely than congenital rubella¹, and 800 times more likely than syphilis.¹ Among the reasons for such neglect could be the fact that the textbooks have never been adequately updated, and that the face of CD itself is believed to be changing.

Associated risks

Common nutrient deficiencies that result from CD include iron, folate, calcium and vitamin D, so CD can be linked with a host of conditions caused by malabsorption of these nutrients.

'Coeliac disease affects the proximal food-exposed intestine,' explains Dr Adrian Cummins, a gastroenterologist with the Queen Elizabeth Hospital in Adelaide. 'Iron is principally absorbed in the duodenum, folate in the jejunum and calcium has active transport-mediated absorption in the duodenum and passive absorption elsewhere along the intestine. Osteoporosis and osteopaenia is caused principally by impaired calcium absorption and less so by vitamin D deficiency,' he pointed out to the *JCM*

It is known that untreated CD in a woman increases her risk of conceiving a child with Down syndrome by more than 100-fold² and increases fertility problems.³ CD increases the risk of spontaneous abortion by 8.9-fold and low birth weight by 5.84-fold.⁴ Because of the seriousness of undiagnosed CD, Italy now includes coeliac screening as part of the routine antenatal but I don't believe that even Australia's IVF clinics routinely perform this simple test. Enquiries made to Sydney IVF to confirm this have to date not even received a response.

In the textbooks, we read about the diarrhoeal pot-bellied child who fails to thrive. Yet nearly 10 years ago, Dr Kamath, a paediatrician from Sydney's Westmead Hospital said that such a child is the lucky one, because he is the one in 10 of all coeliacs who will get diagnosed.⁵ The other nine will continue unwell for years, perhaps their whole lives, without ever being diagnosed. They will face increased risks of thyroid disorders⁶, lymphoma⁷, psoriasis⁸, epilepsy⁹ and other chronic illnesses¹⁰ - risks that might have been reduced had their CD been diagnosed early. Kamath said that all children should be screened at their first school-entry vaccination to prevent some of these complications.⁵

Good studies have shown that type 1 diabetes is associated with increased risk of CD¹¹, and patients with type 1 diabetes should be screened serologically for CD. Many auto-immune diseases are triggered when protein sub-fractions trigger an immune response that then targets tissues with similar amino-acid sequences e.g. streptococcus and heart valves or glomeruli. The susceptibility to these triggers includes the genetic potential or tissue type of the individual. Tissue type is sometimes expressed as the major histocompatibility antigens, or human leukocyte antigen system (HLA). Although abnormal small-intestinal

histology is the gold standard for diagnosis and CD is by definition a primary intestinal disorder, there is growing belief that it is not always only a gut disorder, and some propose that it may bypass the gut altogether.

Epidemiology

A large 1992 study conducted by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, involving 36 centres from 22 countries, found the average incidence of CD to be one in 1000 live births, with a range of one in 250-4000. When the age of diagnosis was included, the predicted rate was one case for every 300 neonates.¹³ In one study, the prevalence of asymptomatic coeliac disease was as high as 1 :266 in blood donors.⁴

However, epidemiological studies presented at the 2000 International Symposium on Coeliac Disease's furnished the following prevalence data:

Italy	1 in 186
Sweden	1 in 180
Finland	1 in 100
Hungary	1 in 85
US	1 in 126

All European studies have confirmed an incidence of at least 1 in 500.

Improved screening tests in the form of tTG IgA, and the ability to screen whole communities of 'low-risk' individuals, has led to awareness that the prevalence of CD is generally 1 in 100, and that 'atypical' clinical presentations are common.

Presentation and diagnosis

Coeliac disease can present with gut symptoms, such as diarrhoea or constipation, or mouth ulcers, proctalgia fugax and Crohn's disease.

Endomysial IgA was once regarded as the most sensitive and specific diagnostic test but this is now replaced by **tissue transglutaminase (tTG) testing**. False positives and false negatives do still occur with this test, at about a rate of 61/1008.¹⁶ However, a recent study that combined tTG testing with referred patients classified as having high risk of CD had 100 per cent specificity, helping to target patients in need of a biopsy.¹⁷

Small-bowel biopsy, provided the patient is consuming gluten, remains the definitive test. Villous atrophy begins in the proximal duodenum and extends distally. Diarrhoea is a poor indicator of CD, and may not develop until several feet of gut is involved. Diarrhoea should thus be seen as the end-stage disease, as months or years of malabsorption may have already occurred. The first part of the duodenum may be spared. Nowadays, gastroenterologists usually take at least four samples at biopsy, starting with the second part of the duodenum, proceeding further down to avoid missing the diagnosis.

Unless the blood level of total IgA is performed, all test results except IgG are invalid. This is because coeliac people have a higher chance of IgA deficiency (varying from 1 :30 to 1 :50, according to the text given) relative to the community norm of 1 :300 to 1 :600. In itself, IgA

deficiency raises one's suspicions because of this simple fact, with data suggesting an approximate 10 per cent predictive value for CD.¹⁸

Recent years have also seen the advent of **HLA DQ genotyping**, where leucocyte (peripheral blood) or buccal-scrape DNA is assessed with polymerase chain reaction. One advantage is that this test is independent of gluten intake. At least 95 per cent of all diagnosed coeliacs are positive for DQ2 or DQ8 genes. The relative risk for CD is greatest for patients who are homozygous for the DQ2 antigen. Comparatively, there is moderate risk for homozygous and DQ8-positive individuals, and minimal risk in non- DQ2 and non- DQ8 individuals.¹⁹

The association of the HLA DQ2 antigen with CD is well established. Although its frequency in Caucasian, North African, Middle Eastern and North Indian populations is around 15-20 per cent, the DQ2 antigen has been found expressed in more than 90 per cent of patients.¹⁶ HLA DQ2 is rare in East and Southeast Asians, Polynesians and indigenous Australians. In the remaining 10 per cent, the majority express the HLA DQ8 antigen. HLA DQ genotyping is a very strong negative predictor if genes encoding HLA DQ2 or DQ8 are absent.

For accurate testing, a patient needs to resume gluten consumption for two months, with a load equivalent to four slices of bread daily. They are always resistant to this challenge, and gene testing can be useful to exclude CD.

New **home-based test kits** (e.g. Biocard Coeliac Disease, AniBiotech; Vantaa, Finland) to screen CD seem to hold some promise, and have been used with a good degree of specificity and sensitivity (93.5 and 96.7 per cent respectively, according to one controlled study²⁰). The benefit of these is apparent if these figures can be maintained. Any claim that endoscopy can be entirely avoided, however, should be viewed with caution at this stage.

Coeliac redefined

Coeliac disease is usually defined as small-bowel mucosal atrophy with improvement or normalisation on a gluten-free diet and a deterioration of the villous morphology during intake of a gluten-containing diet.²¹ The presence of circulating antibodies, and their disappearance on a gluten-free diet, usually supports the diagnosis. This strict definition is more important in infants, where there is a wider differential diagnosis of villous atrophy (cow's milk enteropathy, post-gastroenteritis, giardiasis, common variable immunodeficiency) than in adults.

However, Cummins has proposed that CD is a T-cell-mediated disease due to loss of mucosal oral tolerance.²² As such, the small bowel mucosal cell is merely a spectator to a series of events just below the mucosal surface. The implication that the humoral events may predominate in the normal-bowel latent coeliacs with raised gliadins has led a UK group of neurologists to declare that there may be a group of gluten-sensitive individuals with minimal gut pathology and that the brunt of the disease is borne by the nervous system, analogous to dermatitis herpetiformis.²³

Their comment is that people with circulating antibodies and normal small-bowel biopsy may show a reversal in the ratio of $\alpha\beta$ and ϕ/δ T-populations in the intra-epithelial T-lymphocytes, with no other gut changes.

'There is a separate ill-defined condition of gluten intolerance,' adds Dr Cummins. 'It has a normal biopsy, negative serology and is non- HLA DQ2. It may be due to dietary fructans,' Dr Cummins told the *JCM*.

Genetics

Most, if not all, coeliacs share HLA class II genotypes. This means they have T-cells with the HLA DQ2 or DQ8 haplotype. About 25 per cent of northern Europeans exhibit this genotype but few develop coeliac disease.²⁴ There is only a 70 per cent concordance in identical twins with similar dietary histories^{24,18}, however, Dr Robert Anderson, consultant gastroenterologist in Royal Melbourne Hospital Departments of Clinical Immunology and Gastroenterology, told the *JCM* that updated concordance data indicate 87 per cent is more realistic because twins have been followed longer: 'This is the highest concordance rate for any polygenic immunological disease', he says.

The immunological event in the development of CD is the failure to develop oral tolerance to gluten and this defect is speculated to be linked to the HLA DQ2 or DQ8 gene.

There seems to be almost an absolute requirement that 'coeliac genes' be present to develop CD but less than one in 25 people with the gene seem to do so.

Biochemistry

Gliadins, a.k.a. prolamines due to their high proline and glutamine content, are alcohol-soluble peptide subfractions of gluten. They are found in wheat, rye, oats, barley, triticale and possibly millet. They have characteristic amino-acid sequences, with individual differences according to the grain of origin. These grains are being developed, either by traditional crossbreeding or genetic engineering, to contain maximum gluten. Coeliacs often have other food protein allergies and sensitivities. Milk protein is a common problem for coeliac people²⁵, with similar implications for associated diabetes and thyroid disease.

It has been demonstrated that it is the short amino-acid chains causing the intestinal damage in coeliacs.²⁶ As different genes for CD have been identified, it is becoming apparent that there may be subtle differences between coeliac people as to which aminoacid sequences are problematic. It is thought that there are many hundreds of gluten peptides capable of provoking a T-cell response.

Pathology

The epithelium consists of crypts and villi. Cells differentiate into villous cells as they migrate up the crypt villous axis. Villous atrophy is caused by the cytotoxic effect of lymphocytes on villous enterocytes and derangement of normal architecture. Steroids may affect this, and possibly affect diagnosis.

Distinction must also be made between malabsorption of

macro- and micronutrients. Active vs. passive transport is important. Micronutrients often need to be actively transported against a concentration gradient.

Malabsorption may long precede malabsorption relating to diarrhoea, so coeliacs may present as 'tired all the time' and micronutrient deficient, yet still be overweight and suffering constipation.

Perhaps a more comprehensive pathophysiology involves specific amino-acid sequences being transported to the lamina propria and then being processed by dendritic cells that present them as antigens to CD4 lymphocytes in the heterodimer specific to that oligopeptide. This results in the peptide binding to the HLA DQ

The structure of that heterodimer depends upon the patient's HLA status. On exposure to this oligopeptide, the T-lymphocyte responds with the generation of markers such as IL-2, which result in an inflammatory response involving both cellular and humoral components.

New research indicates that gluten is toxic in CD by an innate immunological pathway, separate from HLA DQ and T lymphocytes.²⁷ Certain peptides in gluten seem to prime the T lymphocytes to react to the CD-causing peptides.

Failure of oral tolerance

Oral tolerance is a fundamental process that ensures that food antigens are recognised by the immune system of the small intestine, but an immune response is suppressed. It begins at weaning and is believed to peak at four-to-six months.

Cytokines involved in oral tolerance include IL-2 receptors, interferon gamma and mast-cell tryptase, which decline after six months despite the presentation of new foods. Breast milk contains a chemical called 'transforming growth factor beta', a natural suppressive agent.

Oral tolerance is such a powerful mechanism that it is essential for survival, and only minor abnormalities of its function are possible. One of these abnormalities is CD. The importance of oral tolerance is just now being appreciated.²⁸

Breastfeeding is protective

The timing of first gluten exposure appears to be important. Introduction of gluten before three months and after seven months both increase the risk, in the former case by fivefold compared to those exposed to gluten at four-to-six months.²⁹

The development of tolerance may take place in a critical window of time, which is assisted by the presence of breast-milk factors. If the first exposure to gluten is under the cover of breast milk, the risk of CD reduces threefold.

Breast milk alone may be insufficient to ensure tolerance, any more than failure to breastfeed automatically condemns the susceptible to developing CD. Breastfed children were at greater risk if the initial gluten exposure took place after weaning and the doses of gluten were high. It is thought that most people who are going to become coeliac do so in the first few years of life, although they may be asymptomatic. Children who have had multiple GI infections seem to be at greater risk, while adults who develop coeliac having earlier been screened negative may do so after a gut infection.

Research on interventions

Four main fronts for treatment of CD are being explored.

- **Digestive enzymes** to help break down gluten. This therapy is designed to complement a gluten-free diet, not replace it.³⁰
- A drug to target **zonulin**. Fasano et al have researched zonulin, a protein that controls the leakiness of gut epithelia.³¹ It increases in all individuals after infection or intestinal inflammation, and is high in coeliacs who are not gluten free.
- **Vaccine/s** to switch off the T-cell response to particular gluten fractions. At least two forms of coeliac need to be targeted: the DQ2 and DQ8 genotypes.
- The development of a **genetically modified wheat** that lacks the peptides known to trigger the immune response/s.³² **Probiotics** are being used in the processing of gliadin-containing foods to make them more tolerable to people with CD.³³
- **HLA DQ2 and DQ8 blocking agents**.
- Inhibitors of **transglutaminase**, an enzyme that deamidates gluten peptides and facilitates their increased binding to DQ2 and DQ8.

Conclusion

The increasing incidence of CD has been accepted as being due to more than simply better diagnosis. It seems to be largely a problem of Western nations, and may represent another manifestation of the hygiene hypothesis [see *JCM2005;4(5):23*].

Vaccination may also play a role. Any factors that hinder the maturation of a Th2 response into a mature Th1 response can result in the inappropriate expression of genes. Both vaccination, and the hygienic protection of babies from normal germs, are outside evolutionary experience.

The gut of a newborn baby from the slums of Lahore teems at three days with bacteria. A Swedish baby may have none at one week of age.³⁴ 'Good' gut bacteria, such as acidophilus and bifidus species, are protective in asthma and eczema, and more than likely in CD as well.

Should we provide newborn with probiotics and even delay vaccination in order to prevent CD, juvenile diabetes, et al?

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