DOES NON-COELIAC GLUTEN SENSITIVITY EXIST?

Thesis for degree of MD

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AGA	Antigliadin Antibody
AOR	Adjusted Odds Ratio
ASA	Acetylsalicylic Acid
ASCA	Anti-Saccharomyces Cerevisiae Antibodies
ATI	Amylase-trypsin Inhibitors
BMI	Body-mass Index
CD	Coeliac Disease
CDAI	Crohn's Disease Activity Index
C.I	Confidence Interval
C-IBS	Constipation predominant Irritable Bowel Syndrome
CLO	Campylobacter-like Organism
D-IBS	Diarrhoea predominant Irritable Bowel Syndrome
DBPC	Double-blind placebo-controlled
DH	Dermatitis Herpetiformis
EMA	Endomysial Antibody
FIS	Fatigue Impact Score
FODMAPs	Fermentable Oligo-, Di-, Mono-saccharides and Polyols
GFD	Gluten Free Diet
GI	Gastrointestinal
GRD	Gluten Related Disorders
GS	Gluten Sensitivity
HADS	Hospital Anxiety and Depression Score
HLA	Human Leukocyte Antigen
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome

IBS-SSS	Irritable Bowel Syndrome Severity Scoring System
IELs	Intraepithelial lymphocytes
Ig	Immunoglobulins
IFN	Interferon
IL	Interleukins
LD	Lymphocytic duodenosis
M-IBS	Mixed pattern Irritable Bowel Syndrome
MRI	Magnetic Resonance Imaging
NCGS	Non Coeliac Gluten Sensitivity
NR	Not Reported
NSAID	Non-steroidal anti-inflammatory drugs
OR	Odds Ratio
PA	Peanut Allergy
PBMC	Peripheral Blood Mononuclear Cells
PPM	Parts per Million
RHH	Royal Hallamshire Hospital
SCAI	Severe Colitis Activity Index
SF-36	Short Form 36
SPSS	Statistical Package for the Social Sciences
SR-NCGS	Self-Reported Non-Coeliac Gluten Sensitivity
TTG	Tissue Transglutaminase Antibody
UK	United Kingdom
USA	United States of America
WA	Wheat allergy
WHO	World Health Organisation
QOL	Quality of life

ABSTRACT

Introduction: Coeliac disease (CD) is a gluten-sensitive enteropathy which affects 1% of the population. The treatment for CD is a strict lifelong gluten-free diet (GFD). IgE-wheat allergy is another gluten-related disorder affecting 0.1-1% of children, although most will have outgrown the condition by adulthood. However, recent media reports suggest increasing popularity and consumption of a GFD even in the absence of CD or IgE-wheat allergy. This has led to the evolution of a newly-defined clinical entity termed non-coeliac gluten sensitivity (NCGS). The aim of this thesis was to determine the existence and characteristics of NCGS.

Methods: We ascertained whether there has been a change in awareness of gluten-related disorders amongst the general public and chefs in Sheffield, United Kingdom. The population prevalence of self-reported gluten-sensitivity and the use of a GFD were also determined, as well as the characteristics and diagnostic outcomes in those patients referred to adult secondary-care gastroenterology practice. Finally, we evaluated whether a GFD is being used independently by inflammatory bowel disease (IBD) patients and also if a GFD can be used to treat patients with diarrhoea-predominant irritable-bowel syndrome (D-IBS) previously naïve to the effects of gluten.

Results: i) There has been a dramatic rise in both the publics' and chefs' awareness of gluten-related disorders over a ten-year period. ii) On questioning 1002 community adults, 13% self-reported gluten-sensitivity with 3.7% consuming a GFD yet only 0.8% having a doctor-diagnosis of CD. Subjects self-reporting gluten-sensitivity were predominantly young to middle-aged women and had a greater prevalence of IBS. Further, a combination of intestinal and extra-intestinal symptoms were described in relation to gluten ingestion;

these include abdominal pain/discomfort, bloating and altered bowel habit (consistent with the criteria used to define IBS), as well as fatigue, headaches, depression, skin rash, and joint pains. On evaluating 200 patients referred to secondary-care with self-reported gluten sensitivity, 7% were found to have CD with the remaining 93% classed as self-reported NCGS. Analysis of those with NCGS suggested that 98.5% could be dietary-related IBS. However, 1.5% were found to have IBD with such patients having demonstrated alarm symptoms and/or abnormal systemic inflammatory markers that necessitated colonic investigations. iii) We then separately established that patients with IBD who self-report NCGS are more likely to have severe or stricturing disease compared to IBD patients who do not report gluten sensitivity. iv) Finally, in D-IBS patients previously naïve to the effects of gluten a six-week trial of a GFD led to clinical improvement in 71% of cases. Furthermore, 72% opted to continue with a GFD thereafter and at 18-month mean follow-up were still taking the diet, maintained symptom improvement, and demonstrated similar anthropometric/biochemical status relative to baseline. We identified that the benefits of a GFD in D-IBS subjects may differ according to the presence or absence of HLA-DQ2/8 genotype.

Conclusion: We have demonstrated that self-reported NCGS does exist. The condition is associated with IBS, although it can rarely be present in IBD. A GFD can be a treatment option in D-IBS patients previously naïve to the effects of gluten. This body of work has significantly contributed towards our understanding of NCGS. However, as with most research projects it has instigated further questions that require exploration.

CHAPTER 1: Irritable bowel syndrome and the spectrum of gluten-related disorders.

1.1 Introduction

Irritable bowel syndrome is a highly prevalent functional gastrointestinal disorder that has a significant impact on quality of life and healthcare resources. Diet is commonly cited as triggering and perpetuating the symptoms of irritable bowel syndrome. One such dietary component is gluten, a protein classically associated with coeliac disease or IgE-wheat allergy. However, there is increasing media coverage to suggest that individuals are now self-reporting gluten sensitive irritable bowel type symptoms and taking a gluten-free diet of their own volition outside a diagnosis of either coeliac disease or IgE-wheat allergy. This clinical entity has been termed non-coeliac gluten sensitivity although its existence remains uncertain and in need of further clarification.

1.2 Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder, as defined by no identifiable structural or biochemical abnormality.[1] Epidemiological surveys suggest that IBS is common with a pooled global prevalence of 11.2%, shows a female preponderance, and is mainly seen in those under the age of 50 years.[2-4] IBS is frequently encountered in clinical practice, accounting for almost a third of all gastroenterology cases seen in primary-care, with a subsequent third of these being referred onto secondary-care for further evaluation.[5]

The burden of illness of IBS is significant. Despite being a benign disorder by definition, IBS leads a chronic remitting-relapsing course with associated fatigue, depression, anxiety, and diminished quality of life (QOL).[6,7] Patients have a decreased health-related QOL scores compared to healthy individuals and even those with chronic disorders, such as diabetes

and end-stage renal failure.[8] In addition, IBS patients generate a substantial economic burden, both due to direct healthcare costs and impaired work productivity.[9] A systematic review addressing the economic cost of IBS in the USA and UK in the year 2002 found total direct and indirect costs per patient per year reaching up to $8750 and $3344, respectively.[10]

Guidelines for the management of IBS recommend the use of symptom based criteria to aid clinicians towards making a positive diagnosis of IBS without the need to perform extensive investigations.[1,11] In 1978, Manning et al first described six key symptoms commonly seen in individuals ultimately diagnosed with IBS.[12] These were later incorporated by a multinational working party to form the Rome I criteria,[13] and have subsequently been revised on two further occasions, to produce the Rome II and, most recently, the Rome III criteria (Table 1).[14-16]

Table 1: The Rome III diagnostic criteria for IBS [15,16]

ROME III diagnostic criteria* for irritable bowel syndrome

Recurrent abdominal pain or discomfort at least 3 days a month in the past 3 months, associated with two or more of the following:

- Improvement with defecation
- Onset associated with a change in frequency of stool
- Onset associated with a change in form (appearance) of stool

*criteria fulfilled for the past 3 months with symptom onset at least 6 months before diagnosis

However, validation of such criteria shows that they perform only modestly in distinguishing IBS from organic diseases.[17] In particular, several studies have now reported that gastrointestinal symptoms in organic conditions can significantly overlap and mimic the symptoms of IBS. These conditions include bile acid diarrhoea,[18-21] exocrine pancreatic insufficiency,[22] inflammatory bowel disease,[23,24] small bowel bacterial overgrowth,[25] and the gluten-related disorder that is coeliac disease.[26]

The pathophysiological mechanism of IBS is not yet completely understood, but is felt to be due to a dysregulation in the brain-gut axis manifested by alterations in the cerebral and autonomic response, immune function, visceral sensitivity and motility.[27] Triggers of such alterations include genetic factors,[28] chronic stress,[29] enteric infections[30,31] and diet.[32]

1.3 The interaction between irritable bowel syndrome and food

An estimated one-fifth of the general population believe they suffer from adverse food reactions.[33] Adverse reactions reported to food can be due to an allergy or an intolerance/sensitivity. In accordance with the Rome Foundation Working Group, an allergy implies a specific immune response (immunoglobulin-E [IgE] or non-IgE-mediated) that occurs reproducibly on exposure to a particular food component.[34] An intolerance or sensitivity is where there is no established immune-mediated reaction as seen for example when posed with the sight, smell, thought or taste of eating unpleasant food. Another example of intolerance is when consuming poorly absorbed carbohydrates that lead to microbial colonic fermentation which in the presence of visceral hypersensitivity can trigger gastrointestinal symptoms.[34-36]

IgE-mediated food allergy is more common in children than adults. Data from the US National Health and Nutrition Examination Survey reported that 4.2% of children under the

age of 5 years had food-specific IgE-serologies.[37] The common allergen proteins in children, accounting for 85% of cases, tend to be cow's milk, egg, peanut, soy, tree nuts, shellfish and wheat.[38] However, with time tolerance develops for many foods including eggs, milk and wheat, although this is less likely with peanuts[38] and has prompted considerable interest in developing prevention strategies for peanut allergy.[39] The prevalence of IgE-mediated food allergies in those aged between 6-19 years is 3.8%, and 1.3% in those over 60 years.[37] Symptoms usually start within minutes, and definitely occur within 2 hours, following exposure to the offending food. These include any combination of oral, skin, respiratory or gastrointestinal symptoms, with the most severe reactions leading to potentially fatal anaphylaxis.[33] First-line testing for IgE-mediated food allergies involves food-specific IgE serologies, and/or skin prick tests, which should be interpreted in the context of the clinical history given that false-positives can occur due to either the development of tolerance following sensitisation or potential cross-reactivity with alternate allergens.[40] If needed confirmatory tests can be performed with oral food challenges in medically supervised allergy departments.[40]

Hence, in adult practice it is infrequent to encounter IgE-mediated food allergy. Nevertheless, perceived adverse food reactions are common in this cohort, particularly in women, with a reported prevalence of 20.4% in the UK community with subsequent double-blind placebo-controlled (DBPC) studies confirming true-sensitivity in around one-fifth of these.[41] These reactions can rather be generally attributed to either food intolerance or non-IgE-mediated reactions. A wide range of systemic symptoms may be experienced related to consumption of the intolerant food, including symptoms compatible with IBS.[41-43] Indeed the converse relationship is also apparent as between 66%-84% of patients with IBS

perceive their symptoms to be related to meals.[44,45] The most common foods implicated tend to be those rich in carbohydrates, gluten, fat, coffee, alcohol, and hot spices.[44,45] Furthermore, those IBS patients who report adverse food reactions tend to have more severe symptoms, associated subjective health complaints of musculoskeletal pains and chronic fatigue, and reduced QOL compared to IBS patient without food sensitivities.[45-47]

Historically, investigators have tried to establish ways of identifying food intolerances with ease so that the cumbersome yet gold-standard method of performing DBPC studies can be avoided. They have shown that IgG-food specific antibodies are more common in IBS subjects than healthy controls,[48] with subsequent dietary exclusion leading to significant clinical benefit.[49,50] For example, in a study conducted in Manchester all 150 unselected IBS subjects had at least one positive IgG-food specific antibody to a panel of 29 dietary food antigens; 87% of patients were positive to yeast, 84% to milk, 57% to egg, 49% to wheat, 49% to cashew nuts, and 39% to peas.[50] Subsequently, patients were randomised to receive one-of-two diets, either eliminating foods to which they carried antibodies (exclusion diet) versus a diet containing foods which they carried antibodies (sham diet). The group given the exclusion diet showed significant improvement compared to the sham diet.[50] However, this study was met with criticism and the use of IgG-food specific antibodies has not been adopted into routine clinical practice,[51] although it is available through commercial laboratories like the York-test. The reasons for these doubts are that all IBS patients had positive IgG antibodies against some dietary antigen,[50] yet it has previously been established that about a third of IBS patients do not have any food intolerances at all.[52] Secondly, up to 87% of IBS patients in Manchester were IgG-positive to yeast yet historically DBPC dietary studies on IBS subjects in nearby Oxfordshire showed 5.5% to be yeast sensitive.[52] This

demonstrates a discrepancy in results and suggests that IgG-based tests lack specificity. Rather it implies that the positive response rate seen with IgG-based dietary exclusion studies is based on the fact that a wide array of potentially intolerant foods are being eliminated together, of whom one or more (but query which) is leading to an improvement in symptoms. Hence, IgG against foods are not recommended as a diagnostic tool,[53] although this area is worthy of future research and would benefit from individualised elimination of food antigens followed by confirmatory DBPC studies.

Nevertheless, as individuals with IBS are keen to seek dietary options to alleviate their symptoms various dietary modifications have been proposed. Unfortunately, many of these have been advertised through media coverage using philosophical or pseudoscientific concepts and without scientific rigour.[54,55] This includes the Paleolithic diet which is based on only consuming foods that were amenable to our ancient ancestors. However, more recently clinical evidence does exist for reducing the intake of carbohydrates known as fermentable oligo-, di-, mono-saccharides and polyols (FODMAPS), which are present mainly in fruit, vegetables, and wheat. These have been shown to alleviate the symptoms of IBS in up to 50-70% of cases through decreasing colonic microbial gas production and preventing osmotic diarrhoea.[56-59] An image of FODMAP containing foods is illustrated as per Figure 1, demonstrating the potential difficulties that may arise if undertaking this extensive diet without expert dietetic input. Elsewhere, individuals are also eliminating gluten from their diet which opens up the possibility to discuss gluten-related disorders.

Figure 1: An example of FODMAP containing foods (from www.google images)

1.4 The evolutionary relationship between gluten and mankind

Gluten is the main storage protein used by some classes of flowering plants to nourish seeds during development and germination.[60] It is a high molecular weight protein found in the endosperm of grass-related grains, including wheat, barley and rye. It is the composite of two classes of protein, a glutenin and a prolamin (gliadin in wheat, hordeins in barley, and secalins in rye), which can be fractionated to produce alpha, beta and gamma peptides. As plant seeds are the plant tissue most consumed by man, seed storage proteins have been long studied and characterized. Wheat gluten was first isolated in 1745[61] and since then further advances in the knowledge of protein structure have established that the prolamin components of gluten are responsible for the ability to process wheat to form dough by means of creating a viscoelastic network.[62,63]

Mankind has existed for about 2.5 million years but cereal crops have been introduced as a component of the human diet about 10,000 years ago during the Neolithic Revolution. This saw a transition from hunting and gathering of food to settled agriculture. The first signs of cultivation have been found in the Fertile Crescent in South West Asia and the subsequent farming expansion lasted until 4,000 BC.[64]

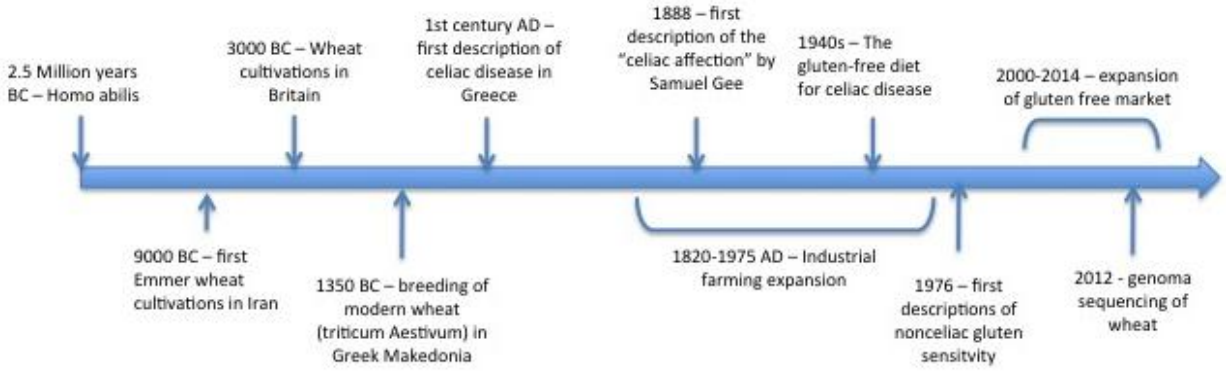
Cereal harvesting and consumption has gradually increased since then, until its major outbreak in the twentieth century. Between the two World Wars, the need to develop a more efficient rationing system and increase agricultural production became evident. The improvement of wheat cultivation became one of the main objectives of the Nutrition Society which was founded in 1941 in Britain to advance the scientific study of nutrition and its application to the maintenance of health.[65] This goal was achieved, with modern day global wheat production amounting to over 700 million tonnes per year (http://faostat.fao.org).

Moreover, the need to ensure an efficient agricultural production has led to the artificial breeding and selection of wheat variants with better adaptation to extreme climate conditions, bread-making qualities and resistance to diseases.[66] This has contributed to a dramatic change in the genetic variety and possibly immunogenic qualities of wheat over time.[66] Currently, about 95% of the wheat grown worldwide is bread wheat (Triticum aestivum), a hexaploid species which resulted from the spontaneous hybridizations between more ancient tetraploid (Emmer) and diploid species (Wild grass) and was then selected by farmers for its superior qualities and yields, such as higher number and bigger seeds.[67] Furthermore, the awareness of the potential role of gluten in processing food has led to the industrial extraction of gluten from plant seeds and its use in the baking industry as an additive with various functions, such as increasing elasticity and stability of food products or as protein supplement to low-protein food.[68]

It is therefore believed that the rate of increase in gluten exposure, from the development of wheat cultivation to modern intensive farming, along with its genetic modification, has been too high to give our immune system the time to develop optimal adaptive

mechanisms, though this "evolutionary theory" has yet to be fully clarified.[69] Nevertheless, perhaps as a result of all these factors have come the changing epidemiology of coeliac disease and other gluten-related disorders (Figure 2).[70]

Figure 2: Timeline of the history of gluten and mankind[70]



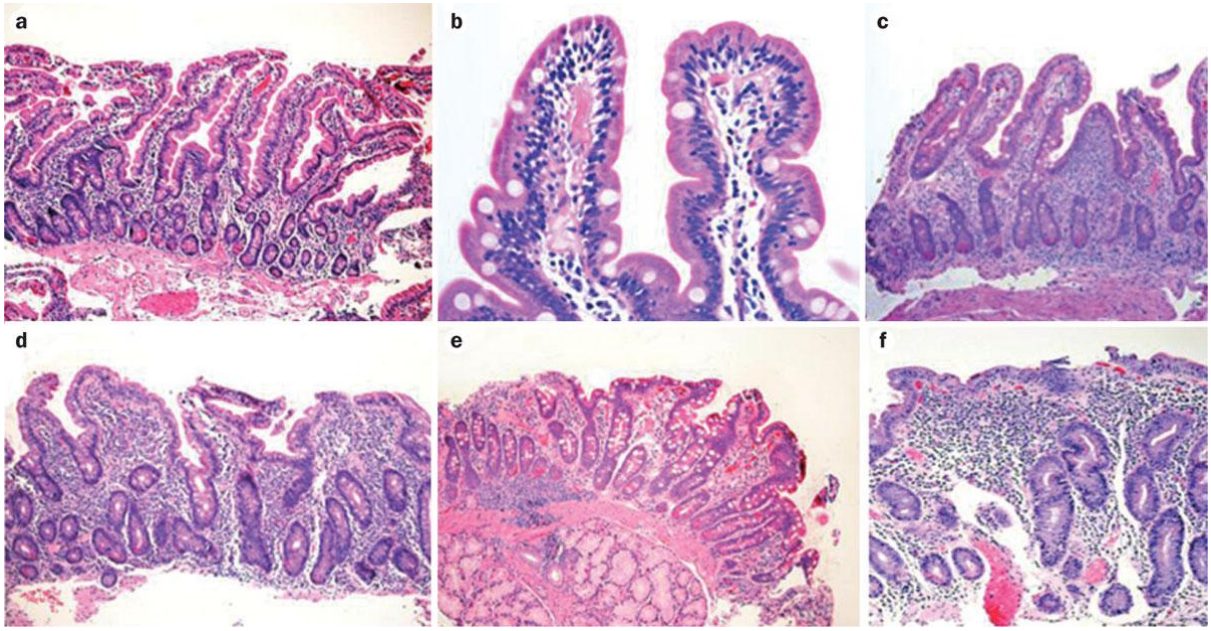
1.5 Coeliac Disease

Coeliac disease (CD), a chronic inflammatory disorder of the small bowel, can be defined as a state of heightened immunological responsiveness to ingested gluten in genetically susceptible individuals.[71,72] All patients with CD carry the human leukocyte antigen (HLA) DQ2 and/or HLA-DQ8 genotypes, although these alleles are present in around 40% of the general population.[73]

The pathogenic pathway that leads to CD consists of a non-IgE-mediated allergic reaction to gluten-derived peptides (gliadin in wheat), coupled with the subsequent development of autoimmunity.[74] Initially, the deamidation of gliadin, by tissue-transglutaminase-2 (TTG-2) an enzyme of the lamina propria, allows it to bind to the HLA-DQ2 and/or DQ8 molecules residing on antigen-presenting cells. The immunogenic epitopes are then presented to CD4+ T-cells which stimulate a cascade of innate and adaptive immune responses. The innate

immune response occurs through activation of macrophages and dendritic cells, via stimulation of receptors such as Toll-like receptor 4. The adaptive immune reaction involves the release of pro-inflammatory cytokines which include tumour necrosis factor-α, interferon-γ (IFN-γ), Interleukins (IL)-6, IL-21, and IL-17. Furthermore, there is also the development of coeliac antibodies, such as those against TTG-2, although the mechanism of autoantibody formation remains incompletely understood.[75] Nonetheless, the subsequent inflammatory reaction leads to step-wise histological damage of the small intestine ultimately culminating as duodenal villous atrophy (Figure 3).[76] The first detectable histological signs are in a rise in duodenal intraepithelial lymphocytes (IELs) of >25 per 100 enterocytes (also known as lymphocytic duodenosis or grade 1 in accordance with the modified Marsh-Oberhuber classification), followed by additional crypt hyperplasia (grade 2), and lastly with villous atrophy (grade 3).[77,78]

Figure 3: Histology of coeliac disease[76]



*Histological slides above: Coeliac disease enteropathy has a wide spectrum of severity that can vary from **a** | and **b** | the mildest infiltration of the epithelium with lymphocytes and preserved villous architecture, to **c** | crypt hyperplasia alone and progressive degrees in villous atrophy from **d** | mild blunting, **e** | moderate villous atrophy.[76]*

Historically, CD was rare with an incidence of 1 in 8000 being reported in the 1950s.[79] However, contemporary epidemiological studies estimate a worldwide prevalence of approximately 1 in 100, or 1%.[80,81] Nevertheless, a considerable proportion of patients still remain undiagnosed with estimates that for every patient diagnosed with CD approximately 5 cases are still yet to be detected.[82] Furthermore, our understanding of the coeliac patient has drastically changed. Whereas previously most cases diagnosed were children it has now been shown that in fact adult cases, characteristically presenting between the fourth to sixth decades, are more frequent occurring at a ratio of 9:1 compared to the paediatric cohort.

In addition, it is now recognised that patients do not always have to present with classical gastrointestinal symptoms of malabsorption but may have non-classical symptoms[83] – these include atypical gastrointestinal symptoms (such as abdominal pain/discomfort, bloating, and altered defecation; seemingly consistent with IBS)[84] or present insidiously such as with iron deficiency anaemia,[85] osteoporosis,[86] ataxia or peripheral neuropathy.[87] Hence, due to symptom overlap, it can be clinically difficult to distinguish CD from IBS.[88]

The diagnosis of CD is based on the demonstration of histological abnormalities on duodenal biopsies, ranging from raised duodenal IELs to villous atrophy, in the presence of positive serology for coeliac antibodies. In the past serum antigliadin antibodies (AGA) were used but in view of their poor diagnostic accuracy for the presence of enteropathy they have generally been superseded by the highly sensitive and specific deamidated gliadin peptide, endomysial (EMA) and TTG-2 antibodies.[89,90]

The cornerstone of treatment for CD is lifelong adherence to a strict gluten-free diet (GFD). The Codex standard which is used in the UK and Europe, and similarly the Food and Drug

Administration in the United States, suggest that foods containing ≤20mg/kg or 20 parts per million of gluten can be labelled as 'gluten-free'. For the majority of patients, a GFD leads to clinical and histological remission, normalisation of standardised mortality rates,[91,92] a reduction in long term health complications (i.e. osteoporosis)[93-95] and in some studies, an improvement in psychological well-being and QOL.[96,97]

1.6 IgE-Wheat Allergy

An IgE-mediated inflammatory response to allergenic proteins contained in wheat and related cereals is the cause of wheat allergy.[98] A wide range of wheat proteins have been implicated including gliadins, glutenins, serpins, thioredoxin, agglutinin, and amylase-trypsin inhibitors. The clinical manifestations exhibited depend upon the route of exposure; direct contact may cause contact urticaria, inhalation causes occupational asthma and rhinitis, and ingestion leads to the traditional food allergy (with skin, gastrointestinal or respiratory manifestations) along with wheat-dependent exercise induced anaphylaxis.[98,99] IgE-mediated wheat allergy may be seen in up to 0.1-1% of children but rarely progresses into adulthood as most will have outgrown the condition.[100] The rates of resolution can be 20% by the age of 4 years, 52% by the age of 8 years, 66% by 12 years, and 76% by 18 years.[101] First-line testing for IgE-wheat allergy is performed using IgE-wheat serology and/or skin prick tests. These should be interpreted in the context of the clinical history given that false-positives may occur due to development of tolerance; such individuals tend to have IgE-wheat levels only slightly greater than normal.[101] If needed confirmatory testing can be performed with oral food challenges in medically supervised allergy departments.

1.7 Coeliac disease and Irritable Bowel Syndrome crossover

The association between CD and IBS was first reported in the year 2001 - sequential patients presenting to secondary-care fulfilling the Rome II criteria for IBS (n=300) were investigated for CD.[26] Participants were initially investigated for CD with serum AGA and EMA. Any participant that had a positive AGA or EMA was offered a duodenal biopsy to confirm the diagnosis of CD. CD was found to be present in 4.7% (14/300) of patients referred to secondary-care fulfilling the ROME II criteria for IBS, a seven fold increase compared to non-IBS matched controls (0.67%), [95% CI 1.6-28.0, p=0.004]. Further analysis were then performed in a population of healthy volunteers recruited from primary-care (n=1200). From 1200 volunteers in primary-care there were 12 new cases of CD. The prevalence of CD in this general population sample was 1% (95% CI 0.4-1.3%). The prevalence of CD amongst patients with IBS in primary-care was 3.3%.[102] These studies highlight the importance of a case-finding approach when considering patients with symptoms of IBS, where the diagnosis of CD may be missed. Since that time others have published supportive evidence/validation studies from other international cohorts (Table 2, page 26).[26,102-117]

A recent systematic review and large meta-analysis found the prevalence of biopsy-proven CD in cases meeting the diagnostic criteria for IBS was more than 4 fold than that in controls without IBS.[118] The recognised association between IBS and CD has led to a change in practice and guidelines. The National Institute for Health and Clinical Excellence in the United Kingdom and the British Society of Gastroenterology guidelines recommend the routine exclusion of CD in all patients referred with IBS.[11,119] The American College of Gastroenterology advice testing for CD in those with diarrhoea-predominant or mixed-bowel pattern IBS (D-IBS, M-IBS).[120] However, this has recently been challenged by Cash et

al who found the prevalence of CD in 492 US patients with non-constipated IBS to be 0.41%, similar to that of healthy controls (0.44%).[117] Given that testing for CD in IBS is cost effective in areas where the prevalence of CD is 1% or greater, this may have implications on future American College of Gastroenterology recommendations. However, this study is the first of its kind in US and further validation studies are required. What adds more to the debate is that a primary-care study in the US noted the prevalence of CD in IBS patients to be 2.7%.[108] It may be that there is an ascertainment bias – in the UK, it is estimated that IBS accounts for at least 25% of a gastroenterologist's workload in the out-patient department.[121] The referral pattern in the US appears to be significantly different to that seen in the UK, as Cash et al recruited 492 patients from 4 centres over 5 years. Does this suggest that IBS is not a condition commonly referred to secondary-care in the US? Perhaps primary-care physicians have already investigated patients for CD prior to referral?[108] In addition, Cash et al and others did not include investigating patients with constipation predominant IBS (C-IBS) for CD as seen in Table 2, yet the largest multicentre CD epidemiological screening study in the US (involving 13,145 patients from 32 states) found CD to be present 2.63% of patients complaining of constipation (40/1530). Furthermore, this group also noted constipation to be prevalent in 20.2% of newly diagnosed CD cases.[122] Therefore, it is not clear whether we should be including or excluding C-IBS patients from identifying CD cases. A standardised method would certainly help elucidate this further as various groups, as shown in table 2, demonstrate diversity in the diagnostic criteria, referral patterns, number of patients, IBS subtypes, serological tests, and histological confirmation that are used to investigate IBS patients for CD.

Moving on, the association between IBS and CD appears to operate in both directions, as patients with CD on a GFD are more likely to describe IBS symptoms than controls. A study by O'Leary et al found 20% of CD patients to also fulfil the ROME criteria for IBS, compared to 5% healthy controls.[123] This study also showed that CD patients with IBS have a markedly lower health-related QOL than their counterparts without IBS. This novel observation is also supported by research from our own department who recently reported in a cross-sectional study (n=1031) that patients with CD and persisting IBS symptoms have worse Short Form-36 (SF-36; a measure of health-related QOL) scores by comparison to those who only have CD.[124]

These patients also report a higher frequency of medical consultations compared to CD non-IBS patients.[125] Predictors of IBS type symptoms amongst adults with CD include mental disorder, female sex, and occasional non-adherence to a GFD.[125] This data offers further support to the biopsychosocial model of IBS, with CD possibly playing its part by having a sensitizing effect on the bowel through mucosal inflammation.

Table 2: Studies of coeliac disease in cohorts of patients with IBS [26,102-117]

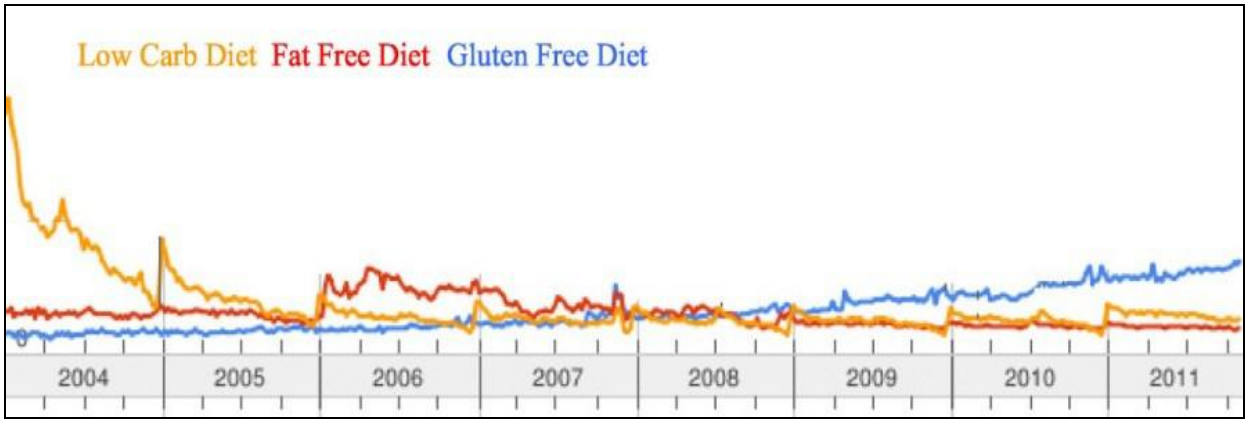
Report	Year	Country	Setting	N	Criteria	IBS subtype investigated (%)	Initial Tests	Biopsy	Prevalence
Hin[103]	1999	UK	Primary-care	132	NR	NR	EMA	Yes	0%
Agréus[104]	2000	Sweden	Primary-care	50	NR	NR	AGA, EMA	Yes	0%
Holt[105]	2001	UK	Primary-care	138	Rome I	NR	AGA, EMA	No	0.7%
Sanders[102]	2003	UK	Primary-care	123	Rome II	NR	AGA, EMA	Yes	3.3%
Locke[106]	2004	USA	Primary-care	50	Manning	D-IBS (38%)	TTG,EMA	No	-
Kennedy[107]	2006	UK	Primary-care	141	Rome I	NR	AGA, EMA	No	0.7%
Catassi[108]	2007	USA	Primary-care	264	NR	NR	EMA, TTG	Yes	2.7%
Sanders[26]	2001	UK	Secondary-care	300	Rome II	D-IBS (28%), C-IBS (21%), M-IBS (51%)	AGA, EMA	Yes	4.7%
Demarchi[109]	2002	Italy	Secondary-care	257	Rome II	NR	AGA, EMA	Yes	8.2%
Shahbazkhani[110]	2003	Iran	Secondary-care	105	Rome II	D-IBS (23%), C-IBS (34%), M-IBS (43%)	AGA, EMA	Yes	11.4%
v d Wouden[111]	2007	Holland	Secondary-care	148	Rome II	NR	EMA	No	0%
Ozdil[112]	2008	Turkey	Secondary-care	60	Rome II	D-IBS (22%), C-IBS (55%), M-IBS (23%)	AGA, EMA, TTG	Yes	0%
Jadallah[113]	2009	Jordan	Secondary-care	742	Rome II	D-IBS (28%), C-IBS (48%), M-IBS (24%)	TTG	Yes	3.2%
Z-Wcisło[114]	2009	Poland	Secondary-care	200	Rome II	D-IBS (100%)	TTG	Yes	7%
Korkut[115]	2010	Turkey	Secondary-care	100	Rome III	D-IBS (21%), C-IBS (63%), M-IBS (16%)	POCT, AGA, TTG	Yes	2%
El-Salhy[116]	2011	Norway	Secondary-care	968	Rome III	NR	D2 biopsy	Yes	0.4%
Cash[117]	2011	USA	Secondary-care	492	Rome II	D-IBS and M-IBS	AGA, EMA, TTG	Yes	0.41%

AGA, antigliadin antibodies; EMA, endomysial antibodies; TTG, tissue transglutaminase antibodies; NR, not reported; POCT, point of care test
D –IBS (diarrhoea), C-IBS (constipation), M-IBS (mixed pattern) predominant irritable bowel syndrome

1.8 The use of a gluten-free diet outside of coeliac disease and IgE-wheat allergy

Until recently the term gluten sensitivity was used synonymously with either CD or IgE-wheat allergy. However, the last 5 years has seen the media report a dramatic shift in the availability and consumption of gluten-free products outside a known diagnosis of CD or IgE-wheat allergy (Figure 4). It has been estimated that 15-25% of US consumers want gluten-free foods and that by the year 2017 the market will be worth some 6.6 billion dollars.

Figure 4: Changing trends of dietary modifications in the USA during the period 2004-11[126]



Y-axis: Compound annual growth rate

1.9 The first case reports of non-coeliac gluten sensitivity

Whether this speculated rise in the use of a GFD is true requires further exploration. Interestingly, on reviewing the literature there are case reports dating back to the mid-1970s of physicians being posed with the clinical dilemma of patients self-reporting gluten sensitivity, and/or taking a GFD, but showing no evidence of CD or IgE-wheat allergy. At the time the term non-coeliac gluten sensitivity was first coined to potentially describe such individuals but did not gain much in the way of further recognition.[127,128] In summary, these brief reports describe a relatively small number of young to middle-aged women complaining of a long-standing and previously unresolved history of abdominal pain, discomfort, bloating, altered bowel habit, and fatigue. They subsequently undergo extensive

gastrointestinal investigations, all of which are negative including the exclusion of CD and IgE-wheat allergy. Their symptoms would seem compatible with the criteria used to diagnose IBS, although the physicians did not state this diagnosis in their report. With various treatment options failing, an empirical trial of a GFD led to a remarkable improvement in clinical symptoms with subsequent relapse on gluten-challenge.[127,128] Following on, one of these groups expanded their findings to publish a paper in 1980 where they describe how in 6 patients, now well controlled on a GFD, double-blinded crossover exposure of gluten-containing flour versus gluten-free flour led to significant symptom induction in the gluten-containing group.[129] However, it is not clear whether participants were able to differentiate between the two challenges based on taste and texture, particularly as the gluten-free flour was commercially available and commonly prescribed for the coeliac diet. Nevertheless, despite the promising findings from this study it proved to be controversial at the time and was met with some scepticism.[130,131] There on, it is difficult to know how such individuals were perceived by their family practitioners or gastroenterologists, but given the significant paucity of further publications in the field for the next 30 years it has been suggested that they may have been left in a "no-man's land" and potentially dismissed as having an underlying psychosomatic ailment accounting for what appeared to be nonsensical gluten-related symptoms.[132]

1.10 Conclusion

The relationship between IBS with CD and IgE-wheat allergy is well established. However, there is increasing media recognition of gluten sensitivity and the use of a GFD outside of the aforementioned gluten-related disorders. The term NCGS has been introduced but given its lack of scientific evidence it can be viewed as a "fertile crescent" for further research.[133]

CHAPTER 2: Hypothesis, aims, methods and collaborations

2.1 Null hypothesis

As outlined in chapter 1 there appears to be increasing media coverage regarding the use of a GFD in the absence of CD or IgE-wheat allergy. This entity could be described as non-coeliac gluten sensitivity. The null hypothesis of this thesis was that gluten sensitivity and the use of a GFD does not exist outside of coeliac disease or IgE-wheat allergy.

2.2 Aims

The null hypothesis has been tested in the following ways:

a) The awareness of gluten-related disorders amongst chefs and the general public has been evaluated over a ten-year period (Chapter 3).

b) The population prevalence of self-reported gluten sensitivity and use of a GFD has been ascertained (Chapter 4).

c) The diagnostic outcome of patients presenting to secondary-care with self-reported gluten sensitivity has been determined (Chapter 4).

d) The existence of self-reported gluten sensitivity in inflammatory bowel disease has been evaluated (Chapter 5).

e) The benefits of recommending a GFD in patients with diarrhoea-predominant IBS previously naïve to the effects of gluten has been determined (Chapter 6).

2.3 Methods

2.3.1 Subject recruitment

Specific participant recruitment for each of the studies is detailed in individual chapters. Patients were given information leaflets and adequate time to consider their participation in the respective studies.

2.3.2 Methods and statistical analysis

Methods and statistical analysis are detailed in individual chapters. All calculations were performed using Statistical Package for the Social Sciences (SPSS) versions 19 to 21. All p values provided are 2 sided with a p value <0.05 considered significant. Advice and support for statistical analysis was provided by staff at MASH (Maths and Statistical Help) through the University of Sheffield.

2.4 Collaborations

As with all research this was a collaborative process. I am extremely grateful to the clinicians and numerous medical students who helped contribute towards the chapters. Chapter 3: Dr Mohammad Karajeh, Jossie Zilkha, Euan Tubman and Charlotte Fowles. Chapter 4: Dr Nina Lewis, Professor Marios Hadjivassiliou, Stefanie Winfield, Nathan Rugg and Laurence Newrick. Chapter 5: Dr Federica Branchi, Katherine Pearson and Josephine Priest. Chapter 6: Jonathan North, Nick Trott and Rebecca Briggs. Finally this would not have been possible without the unflinching support, mentorship and supervision of Professor David Sanders.

CHAPTER 3: Awareness of gluten-related disorders amongst chefs and the general public in the United Kingdom: a 10 year follow-up study

3.1 Summary

Background & Objectives: In view of the rising media coverage regarding the gluten free-diet (GFD) we sought to determine whether there has been a change in awareness of gluten-related disorders (GRD) amongst the general public and chefs. **Methods:** A face-to-face questionnaire about coeliac disease (CD) and gluten sensitivity (GS) was performed on the general public and chefs based in Sheffield, United Kingdom. The assessment was first conducted in 2003 and repeated in 2013. **Results:** In total, 513 public members in year 2003 (mean-age 49.2 years, 62% female) were compared to 575 public members in year 2013 (mean-age 37.8 years, 57% female). There was a significant rise in the publics' awareness of GRD from the years 2003 to 2013; CD (44.2% to 74.4%, adjusted odds ratio (AOR) 3.9; C.I 3.0-5.19) and GS (58.3% to 89%, AOR 7.1; C.I 5.0-9.98), p-value<0.001. Also, 322 chefs in year 2003 (mean-age 37.6 years, 15% female) were compared to 265 chefs in year 2013 (mean-age 27.1 years, 38% female). There was a significant rise in chefs' awareness of GRD from the years 2003 to 2013; CD (17.1% to 78.1%, AOR 12.5; C.I 7.9-19.6) and GS (9.3% to 87.5%, AOR 65.7; C.I 35.4-122), p<0.001. Whereas in 2003 the public were significantly more aware of GRD than chefs, by 2013 this had reached similar prevalence in both groups. In addition, the correct recognition of the gluten-free symbol was 44% for the public and 40% for chefs (p=0.28). Gluten-free products were sold by 41% of restaurants and 27% of takeaways (p=0.07). **Conclusion:** There has been a dramatic rise in both the public and chefs awareness of coeliac disease and gluten sensitivity. Such findings may also ease the social phobia that individuals with GRD have traditionally been accustomed to.

3.2 Introduction

The cornerstone of treatment for coeliac disease (CD) is a strict, lifelong, gluten-free diet (GFD) which in the majority leads to clinical and histological remission, normalisation of standardised mortality rate, and a reduction in long term health complications.[91-97]

Adhering to a GFD has historically been associated with a negative impact on quality of life, particularly in the economic and social domains.[134-137] Studies have shown that gluten-free products are of limited availability and come at a higher cost than their standard counterparts.[134,135] Furthermore, a United Kingdom study has previously revealed that awareness of CD amongst members of the general public and chefs to be lacking.[138] These restrictions have justifiably led to CD patients reporting a greater prevalence of social phobia than healthy controls, and tending to eat food prepared at home rather than dine out.[137-143] In fact, between 60-70% of CD patients consider a GFD to negatively impact on their social activities and dining out, with up to 25% of those having CD for 2-5 years choosing not to dine out at all.[136]

Yet, over the last few years, media reports suggest a noticeable rise in the use of a GFD outside a diagnosis of CD. The term non-coeliac gluten sensitivity has been mentioned although there is a significant paucity of scientific evidence. To address this further we assessed whether there has been a change in awareness of gluten-related disorders (GRD) amongst members of the general public and chefs in the UK.

3.3 Methods and Materials

Participants and study design

A questionnaire survey (Appendix A and B) enquiring about knowledge of CD and gluten sensitivity (GS) was performed in Sheffield, UK. The study was first performed in 2003,[138] and repeated a decade later in 2013. In both time-periods the groups were selected from the same geographical and shopping areas in Sheffield, which was within the central hub of the city. Adult members of the general public, aged over 16 years, were individually approached as they entered or exited several large local shopping areas. Chefs were questioned whilst working in restaurants and takeaways.

The interviews were conducted by means of a face-to-face structured questionnaire. Basic demographic information was obtained following which the groups were asked if they had heard of GS and CD. In addition, the groups were also asked if they had heard of peanut allergy (PA), a condition with prevalence similar to that of CD, at around 1%, but perhaps of greater familiarity in view of its immediate and potentially catastrophic complications.[144] Chefs were also subjectively asked if they were formally qualified but were not asked to provide any objective proof of their education, courses, qualifications or training certificates.

Additional questions, for both groups in the year 2013, was the correct recognition of the cross-grain symbol signifying gluten-free products (Figure 5). For the chefs in 2013, they were also asked whether they displayed any signs or notices selling gluten-free products.

Figure 5: The cross-grain symbol signifying gluten-free products *(picture obtained from Coeliac UK website)*

To assess whether there has been a change over time, comparisons were made between chefs and the general public in the year 2003 against those in the year 2013.

Statistical analysis

Statistical analysis was carried out by using SPSS version 19.0 software. Continuous variables were summarized by mean and standard deviation (SD), with differences between two groups calculated using the Student T-test. Categorical variables were summarized by descriptive statistics, including total numbers, percentages and associations between unadjusted rates analyzed by the chi-squared test. Logistic regression analysis, whilst controlling for potential confounders, were employed to further distinguish between the groups in the year 2003 against the year 2013. Adjusted odds ratios (AOR) and 95% confidence intervals (95% C.I) were determined, with a p-value <0.05 considered statistically significant.

Ethics

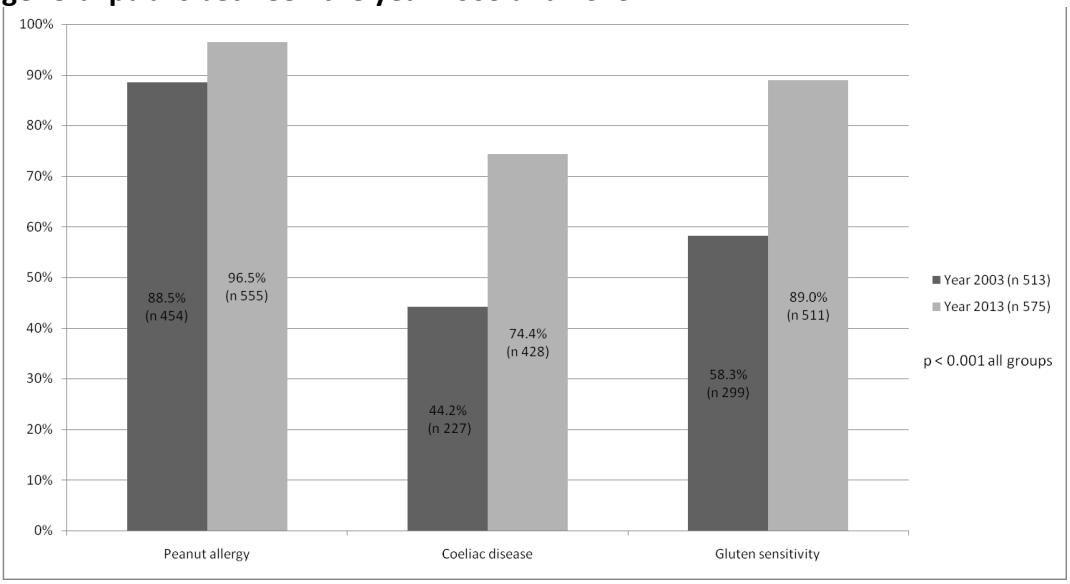
The study was registered with Sheffield Teaching Hospitals research department and conducted according to principles of the Declaration of Helsinki and Good Clinical Practice Guidelines.

3.4 Results

Change in awareness amongst the general public

513 public members (mean-age 49.2 years, 62% female) in the year 2003 were compared against 575 public members (mean-age 37.8 years, 57% female) in the year 2013; p-value for age <0.001 and gender 0.1. There was a rise in awareness of PA (88.5% to 96.5%), CD (44.2% to 74.4%) and GS (58.3% to 89%) in public members from the year 2003 to 2013 as seen in Figure 6. After adjusting for age and gender this was significant amongst all groups with a p<0.001; for PA (AOR 4; 95% C.I 2.3-6.9), for CD (AOR 3.9; 95% C.I 3.0-5.19) and GS (AOR 7.1; 95% C.I 5.0-9.98).

Figure 6: Awareness of peanut allergy, coeliac disease and gluten sensitivity amongst the general public between the year 2003 and 2013

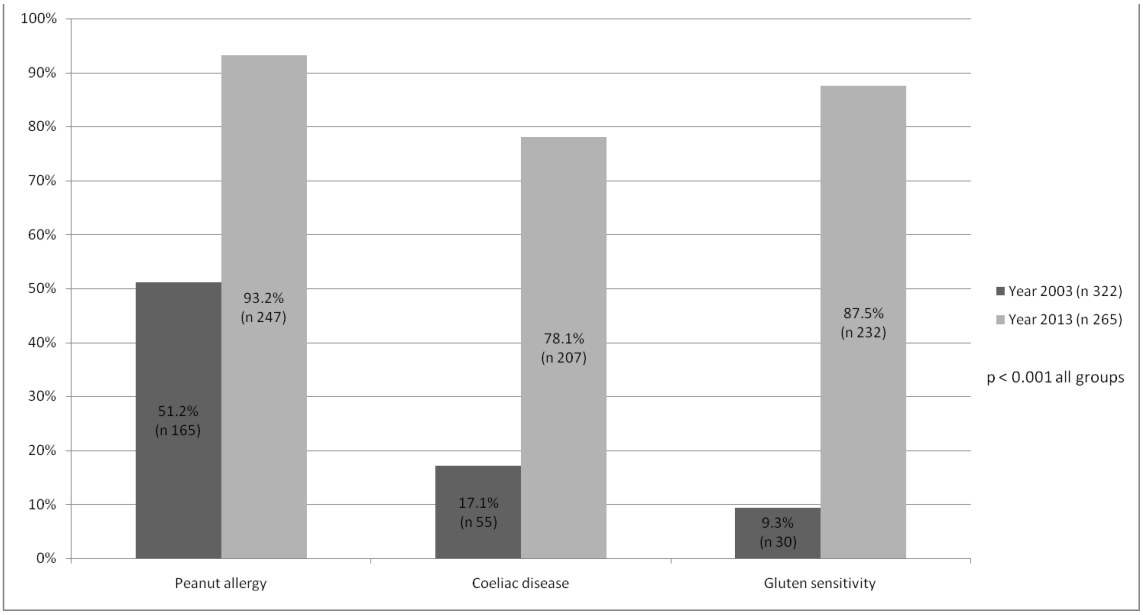


Change in awareness amongst chefs

322 chefs (mean-age 37.6 years, 15% female) in the year 2003 were compared against 265 chefs (mean-age 27.1 years, 38% female) in the year 2013; p-value for age and gender <0.001. A greater percentage of the year 2013 chef group worked in restaurants compared to the year 2003 group (83% vs. 50%, p<0.001), although there was no difference in the rate of qualifications between the two groups (52.5% vs. 52.2%).

There was a rise in awareness of PA (51.2% to 93.2%), CD (17.1% to 78.1%) and GS (9.3% to 87.5%) from the year 2003 to 2013 as seen in Figure 7. After adjusting for age, gender, qualifications and workplace this was significant amongst all groups with a p<0.001; for PA (AOR 8.9; 95% C.I 5.1-15.7), CD (AOR 12.5; 95% C.I 7.9-19.6) and GS (AOR 65.7; 95% C.I 35.4-122).

Figure 7: Awareness of peanut allergy, coeliac disease and gluten sensitivity amongst chefs between the years 2003 and 2013



Comparison of the general public vs. chefs

As previously reported, in the year 2003 the public were more likely to have heard of the three different conditions compared to chefs; PA (88.5% vs. 51.2%, AOR 9.2), CD (44.2% vs. 17.1%, AOR 4.9) and GS (58.3% vs. 9.3%, AOR 13.2).[138]

However, by the year 2013 the knowledge amongst the general public and chefs was of similar prevalence; PA (96.5% vs. 93.2%), CD (74.4% vs. 78.1%), GS (89% vs. 87.5%) and GFD symbol recognition (44% vs. 40%, p=0.28).

Factors associated with awareness of GRD

Subgroup analysis of the general public in year 2013 shows that, in summary, women and increasing age appear to be factors associated with a greater awareness of GRD and recognition of the GFD symbol (Table 3).

Table 3: Factors influencing awareness of peanut allergy and gluten-related disorders amongst the general public in year 2013

Public	Heard of PA	Heard of CD	Heard of GS	Recognize GFD symbol
Female : male (%)	98 : 95	83 : 63	90 : 87	50 : 36
P-value	0.11	< 0.001*	0.22	0.001*
Age group (%) (years)				
≤ 20	96	70	77	33
21–30	95	69	87	36
31–45	96	78	91	57
46–60	97.6	80	95	52
> 60	100	76	94	42
P-value for trend	0.8	0.06	< 0.001*	0.01*

CD, coeliac disease; GFD, gluten-free diet; GS, gluten sensitivity; PA, peanut allergy.
*Statistical significance.

However, such factors did not influence awareness of GRD in the chef group, although female chefs were more likely to recognise the GFD symbol compared to their male counterparts (Table 4).

Table 4: Factors influencing awareness of peanut allergy and gluten-related disorders amongst chefs in year 2013

Chefs	Heard of PA	Heard of CD	Heard of GS	Recognize GFD symbol
Female : male (%)	97 : 91	83 : 75	92 : 85	50 : 34
P-value	0.05	0.12	0.08	0.01*
Age group (%) (years)				
≤20	97	85	89	33
21–30	91	73	88	42
31–45	89	68	80	48
45–60	88	88	96	44
P-value for trend	0.27	0.35	0.9	0.12
Qualified : nonqualified (%)	96 : 90	81 : 75	91 : 84	45 : 35
P-value	0.1	0.19	0.11	0.11
Restaurant : takeaway (%)	94 : 89	79 : 73	90 : 78	43 : 24
P-value	0.21	0.4	0.03*	0.02*

CD, coeliac disease; GFD, gluten-free diet; GS, gluten sensitivity; PA, peanut allergy.
*Statistical significance.

Furthermore, comparing restaurant chefs against takeaway chefs revealed no difference in awareness of PA or CD, but a greater awareness of GS and recognition of the GFD symbol amongst restaurant workers. Restaurants were also more likely to sell gluten-free products than takeaways (41% vs. 27%), although this did not quite reach statistical significance (p=0.07).

3.5 Discussion

This study has established a change in awareness of GRD amongst the general public and chefs over a ten year period. Whereas a decade ago knowledge of GRD appeared limited, particularly amongst chefs, it has now significantly increased and is of similar prevalence in both chefs and the general public.

These findings may be a reflection of the increasing media coverage highlighting the popularity of gluten-free products, thereby providing a constant influx of information.[145] Additionally, individuals working in the food industry are regularly faced and educated by such demands through their consumers. Further sources of knowledge for chefs may also be acquired through their current training curriculum (although no difference was detected between qualified vs. non-qualified) plus being aware of recent changes in Food Standard Agency regulations. Historically, only pre-packaged foods were labelled with ingredients and allergenic foods. However, since December 2014 new legislation (the European Union Food Information for Consumers Regulation 1169/2011) requires food businesses to also provide allergy information on food sold unpackaged, in for example catering outlets, deli counters, bakeries and sandwich bars. Both peanuts and gluten comprise two of the 14 allergens that must be clearly stated if present in the food.[146] The fact that gluten is mentioned as an allergen, rather than an intolerance or sensitivity, may also be a reason why it has gained such recognition and become almost as familiar as PA. In light of these changes in food standard regulations it can be anticipated that knowledge for chefs will continue to grow.

Our findings will also be of welcome news to patients with GRD, in particular those with CD, who have long suffered with fear from dining out. As eating is more than just a physiological

process of meeting an individual's nutritional needs, but also a means of social interaction, the experiences of CD patients have previously shown that they feel negatively controlled by food, with the diet impacting on their emotions, relationships and day-to-day management.[139] They report isolation, shame, widespread ignorance, fear of contamination, being a bother and as a result some have avoided disclosure of their condition and subsequently taken risks when having to eat out.[136-143] Despite strict adherence at home, a large proportion of individuals admit to intentional dietary indiscretions when away from home; 81-88% at social events, 82-88% at restaurants and 58-67% with friends.[136] These violations inevitably lead to ill-health, and may account for persisting chronic inflammation which can be seen on duodenal biopsies in those who may have had the disease long-term.[147] It may now be that with the rise in awareness of GRD, and the GFD, individuals with such disorders no longer need to feel that their disease is a burden and intrusive to their daily life, but can take greater comfort and confidence dining out with the knowledge of being able to discuss their options with chefs. This may lead to a significant improvement in their QOL.

However, caution must still be taken despite chef's awareness of GRD and the availability of gluten-free food. A recent Irish study noted that staff acknowledgement, gluten-free notices, signs and menu were not an absolute guarantee of risk-free dining.[148] Of the 258 premises visited throughout Ireland, 260 food samples were purchased on the assurance that it did not/probably did not contain gluten. Whilst the majority of these were found to be "gluten-free" containing ≤20mg/kg of gluten (90%), or contain "low-levels of gluten" between 21 and 100mg/kg gluten (2.3%), a gluten load >100 mg/kg was encountered in 7.7% of samples. Staff hesitation during interaction with the sampler, and a lack of gluten-

free signage, were the main determinants of encountering gluten in a supposedly gluten-free meal.

Hence, individuals with GRD may still seek further assurances and clarity when eating out. One method of addressing such anxieties can be through food service outlets being formally recognized for their excellence in gluten-free catering. In the UK, this can be obtained through the Coeliac UK accreditation scheme, a recent establishment by the national charity that has led to increased consumer confidence and has had a positive impact on businesses.[149] Accreditation is granted to venues confident that their gluten-free offerings contain ≤20mg/kg gluten and successfully pass an external audit. Other caterers, who remain committed to providing for people with GRD and can demonstrate their knowledge and experience in this field but do not wish to make claims over gluten levels can opt for Coeliac UK's "No Gluten-Containing Ingredients" accreditation. Prior to any form of accreditation, the charity offers online and face-to-face training. Since its enrolment in 2012, more than 1800 establishments have been awarded accreditation.[149] A similar scheme, known as the gluten-free certification programme, is now also in operation in North America.[150]

There are limitations to this study. We note that there was a demographic mismatch in age and gender between the groups. With regards to the general public, increasing age and female gender were associated with greater awareness of GRD. This could have potentially affected our results although any ascertainment bias was avoided on two grounds; firstly, statistical analysis controlled for any confounding variables and secondly, the general public group in year 2013 (compared to 2003) actually comprised a significantly younger cohort, with similar female prevalence, yet showed a marked increase in awareness of GRD. It was

also noticeable that the number of female chefs had more than doubled over the ten year period, although age or sex did not affect awareness of GRD in chefs. As there was no difference in the selection and sampling process, we feel that the greater preponderance of female chefs may be reflective of a societal trend in workforce. Finally, whether our results can be generalised to other cities in the UK, and in Europe, is yet to be determined. Data from the United States does suggest that awareness of GRD is common in New York City.[151] However, this may have partly been augmented by the New York Health Department recently authorising allergen awareness posters to be placed in restaurants throughout the city.[151] It remains to be elucidated whether such results can be replicated in other American states, many of whom do not have such promotional campaigns.

3.6 Conclusion

This study has shown that knowledge of coeliac disease and gluten sensitivity has significantly increased over the last decade amongst both members of the general public and chefs. This appears to coincide with the increasing media coverage highlighting gluten-related disorders.

CHAPTER 4: A United Kingdom study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary-care

4.1 Summary

Background: Media reports suggest gluten sensitivity (GS) exists in the absence of coeliac disease (CD) and IgE-wheat allergy (WA). Following recent double-blind placebo-controlled studies this clinical entity has now been termed non-coeliac gluten sensitivity (NCGS). **Objectives:** To determine the adult population prevalence of self-reported GS and referral characteristics to secondary-care. **Methods:** A UK population-based questionnaire screened for GS and related symptoms. Diagnostic outcomes were also analyzed in patients referred to secondary-care with GS, with tests being performed for CD and WA. A diagnosis of NCGS was based on exclusion of CD and WA. Clinical comparisons were made between NCGS and CD. **Results:** 1002 adults in the population (female 55%, mean-age 39yrs). The self-reported prevalence for GS was 13% (female 79%, mean-age 39.5yrs), with 3.7% consuming a gluten-free diet and 0.8% known to have a doctor-diagnosis of CD. Subjects with GS had an increased prevalence of fulfilling the Rome III criteria for irritable bowel syndrome (IBS), in comparison to those without GS (20% vs. 3.89%, OR 6.23, $p<0.0001$). In secondary care 200 GS patients (female 84%, mean-age 39.6yrs) were investigated, in whom 7% were found to have CD and 93% NCGS. All CD patients were HLA-DQ2 and/or DQ8 positive compared to 53% of NCGS cases ($p=0.0003$). Nutritional deficiencies ($p\leq0.003$), autoimmune disorders (23.1% vs. 9.7%, $p=0.0001$) and a lower mean body mass index (23.7 vs. 25.8, $p=0.001$) were significantly associated with CD compared to NCGS. **Conclusions:** GS is commonly self-reported with symptoms suggesting an association with IBS. The majority of patients do not have CD or WA and would therefore be considered as NCGS, an entity which demonstrates clinical and immunologic difference to CD.

4.2 Introduction

4.2.1 The emerging evidence for non-coeliac gluten sensitivity

With growing reports suggesting gluten sensitivity may exist in the absence of coeliac disease (CD) or IgE-wheat allergy (WA) various investigators have now undertaken double-blind placebo-controlled (DBPC) studies to ascertain the nature of this relationship. Importantly, from the outset when interpreting these studies it needs to be borne-in-mind that gluten is only one of the complex milieu of nutrients present in wheat and other constituents are also capable of triggering symptoms.[152] For example, fermentable oligo-, di, mono-saccharides and polyols (FODMAPS) can cause gastrointestinal symptoms compatible with IBS through gaseous production and osmotic diarrhoea,[56-59] and are present in many food products, with fructans commonly present in wheat.[153]

Carroccio et al recruited 920 Italian adults with IBS who self-reported gluten-based sensitivity without evidence of CD or WA. Following four-weeks of a dietary-elimination period patients underwent a DBPC challenge of receiving either wheat or xylose capsules for 2 weeks followed by a 1 week washout followed by receiving the other capsule for another 2 weeks (crossover design).[154] The investigators found that 30% (n=276) reacted to the wheat challenge which induced symptoms of abdominal pain, bloating and altered stool consistency. Furthermore, two distinct groups were identified; those with wheat sensitivity alone (n=70) and those with wheat sensitivity associated with multiple food sensitivities (n=206).[154,155] However, despite these novel findings, the main limitation of this particular study is that wheat was used making it impossible to differentiate whether it was gluten or an alternate constituent in wheat evoking symptoms.

Biesiekierski et al recruited 34 Australian patients with IBS who self-reported gluten sensitivity.[156] They ensured adequate exclusion of CD as demonstrated by negative HLA-DQ genotypes or normal duodenal biopsies on a gluten-containing diet in those individuals expressing the HLA-DQ genotypes. Thereafter, participants were symptomatically controlled on a GFD and underwent a DBPC challenge of receiving snacks of either 16g of gluten per day or placebo, in the form two bread slices and one muffin daily for up to six weeks. The snacks were prepared to be free of FODMAPS and were the same in taste and texture. Subsequent visual analogue scores revealed that within the gluten-group 68% (n=13/19) reported that their symptoms were inadequately controlled compared to 40% (n=6/15) in the placebo group; p=0.0001. Patients were significantly worse with gluten within one week for overall symptoms, abdominal pain, bloating, stool dissatisfaction and tiredness.

Following this, 15 international experts in the field of gluten-related disorders produced a consensus document in the year 2012 where a new clinical entity termed non-coeliac gluten sensitivity (NCGS) was introduced and embedded into the medical dictionary.[126] Owing to the absence of diagnostic biomarkers, NCGS has been defined as gluten-related symptoms without evidence of CD or WA.[126,157]

However, soon after the publication of the expert consensus document the existence of NCGS was called into question by a subsequent study which instead implicated that non-gluten components, specifically FODMAPs, might be responsible symptom triggers.[158] The investigators found that individuals with self-reported NCGS already on a GFD further benefitted when openly placed on a low-FODMAP diet.[158] Furthermore, the 37 participants in this study then underwent a DBPC crossover trial whereby they received high-dose gluten (16 g gluten per day), low-dose gluten (2 g gluten and 14 g whey protein per day) or control

(16 g whey protein per day) for 1 week followed by a washout period of at least 2 weeks before switching to the next diet. The investigators found no specific or dose-dependent effect of gluten.[158] However, recruitment for this study was through media advertisement and many of the individuals presenting with self-reported NCGS were still symptomatic whilst on their GFD, recording visual analogue scale ratings of up to 60; this finding might not be reflective of those who truly have NCGS. Also, the DBPC crossover trial showed a nocebo response amongst the three arms, which suggests an anticipatory effect of the crossover study design.

Since, two other groups have tried to overcome these uncertainties by performing DBPC studies using wheat gluten, with FODMAPS having been eliminated. Di Sabatino et al showed that small amounts of purified wheat gluten can trigger symptoms in self-reported NCGS; in a DBPC crossover trial involving 59 participants, intake of 4.375 g of gluten per day for 1 week via gastro-soluble capsules significantly increased overall clinical symptoms compared with placebo in the form of rice starch ($p=0.034$).[159] Intestinal symptoms such as abdominal bloating and pain, and extra-intestinal symptoms such as foggy mind, depression and aphthous stomatitis, were significantly more severe in individuals who received gluten than in those who received placebo.[159] Shahbazkhani et al performed a DBPC study where 72 patients with IBS, who were well controlled on a GFD, were randomised to either six-weeks of 50g of gluten-containing powder/day (n=35) or gluten-free powder/day (n=37). There was a significant symptom deterioration in 74.3% of the gluten-containing group, compared to 16.2% of those receiving gluten-free powder.[160] An up-to-date summary of all the DBPC dietary intervention studies in self-reported NCGS is provided in table 5.

Table 5: Studies evaluating DBPC dietary interventions for self-reported NCGS and IBS

Study (Year)	Population	Design	Intervention	Response
Cooper et al (1980)[129]	6 UK patients with IBS-type symptoms who responded to a GFD	DBPC crossover trial	GFD plus either 20g gluten-containing flour or gluten-free flour for the first 3 days of weeks-2 and 4	Significant overall worsening of intestinal symptoms for gluten containing group (p=0.0025)
Biesiekierski et al (2011)[156]	34 Australian patients with IBS (Rome III) & self-reported NCGS	DBPC re-challenge trial	GFD plus either FODMAP-free gluten supplement (muffins and bread; 16 g/day) or similar placebo for 6 weeks	68% gluten vs. 40% placebo worsening of symptoms; p=0.0001 (pain, bloating, stool consistency, and tiredness);
Carroccio et al (2012)[154]	920 Italian patients with IBS (Rome II) and self-reported NCGS	DBPC crossover trial	4 weeks elimination diet followed by DBPC challenges using capsules containing wheat or xylose for 2 weeks (washout period of 1 week)	30% of patients had reappearance of symptoms on wheat challenge.
Biesiekierski et al (2013)[158]	37 Australian patients with IBS (Rome III) and self-reported NCGS	DBPC crossover trial	a) 2-weeks run-in period on a diet low in FODMAPs. Continued thereafter b) 1 week challenge: 1 of 3 diet treatments High-gluten [16g/day] Low-gluten [2 g/day] Placebo (washout period of 2 weeks minimum) c) 3-day re-challenge trial: 1 of three diet treatments (washout period of 3 days minimum)	a) Most patients improved on a low FODMAP diet (p< 0.001) b) Re-appearance of symptoms: High-gluten, 16%; Low-gluten, 3%; Placebo, 8% (p= NS). c) No differences between groups.
Di Sabatino et al (2015)[159]	59 Italian patients with self-reported NCGS	DBPC crossover trial	GFD plus either 4.375g/day FODMAP-free purified wheat gluten or rice-starch placebo for 1 week, via gastrosoluble capsules. (washout period 1 week)	Overall worsening of symptoms with gluten, compared to placebo; p=0.034 (bloating, abdominal pain, foggy mind, depression, aphthous stomatitis)
Shahbazkhani et al (2015)[160]	72 Iranian patients with IBS (Rome III) improving on a GFD	DBPC re-challenge trial	GFD plus either 50g/day FODMAP-free gluten-containing powder or gluten-free powder with 150ml warm water for six-weeks	Overall worsening of intestinal symptoms with gluten compared to placebo; 74.3% vs. 16.2%, p<0.001
Zanini et al (2015)[161]	35 Italian patients with self-reported NCGS on a GFD	DBPC crossover trial	GFD plus either gluten-containing flour (FODMAP-negative) vs. gluten-free flour (FODMAP-positive). 10 days per diet with a 2-week washout period in between.	34% symptomatic with gluten-containing flour 49% symptomatic with gluten-free flour 17% no response

IBS, irritable bowel syndrome; DBPC, double-blind placebo-controlled; GFD, gluten-free diet; FODMAPs, Fermentable oligo-, di-, monosaccharides and polyols; NS, non-significant

These studies suggest that NCGS is a heterogeneous entity in which the culprit agent may be due to gluten in some and fructans (FODMAPS), or placebo, in others. Indeed, the most recent DBPC by Zanini et al illustrates this complexity after identifying 34% of patients with self-reported NCGS to be sensitive to gluten, with 49% not to be gluten sensitive but symptomatic possibly due to FODMAPs instead. In 17% of cases there was no response to either challenge.[161]

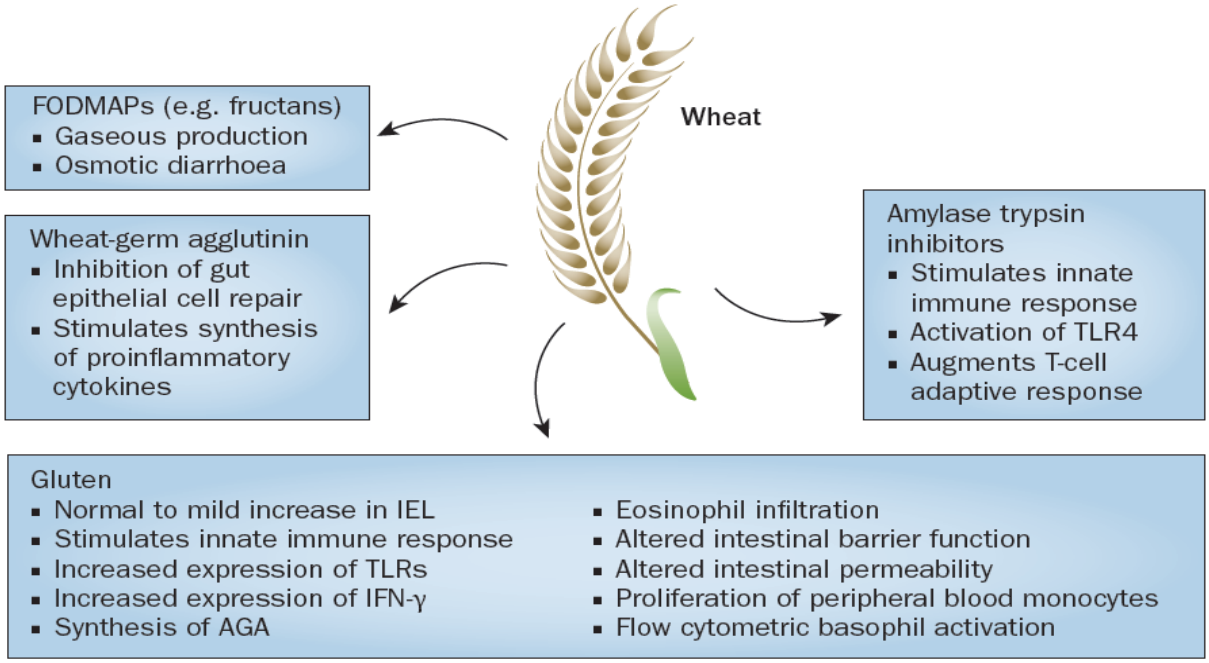
Furthermore, despite the efforts of the aforementioned high-quality DBPC studies in attempting to discriminate gluten from fructans, novel insights have since revealed that other proteins co-exist alongside gluten which may not have been extracted from the purified wheat-gluten used. These include wheat-germ agglutinins (lectins) and amylase-trypsin inhibitors (ATI) that are able to trigger innate immunity, and therefore may have a role in the development of symptoms after ingestion of cereals.[162-165]

ATIs are natural pesticides that account for ~2-4% of the protein content in wheat. Both *in vivo* and *in vitro* studies have shown ATIs to induce an innate immune reaction through activation of Toll-like receptor 4 on monocytes, macrophages and dendritic cells, leading to release of proinflammatory cytokines within 2-12 h.[162] Furthermore, biopsies from patients with CD demonstrate that ATIs augment the gluten-specific T-cell adaptive response.[162] However, mouse models that are deficient in Toll-like receptor 4 or its signalling pathway are protected from immune responses upon oral ingestion of ATIs.[162] Therefore, ATIs have been identified as potential new players fuelling inflammation in both CD and NCGS.[166] Furthermore, wheat-germ agglutinin is a carbohydrate-binding protein that also functions as a natural pesticide. Preliminary studies demonstrate that they can inhibit repair of gut

epithelial cells and also stimulate the synthesis of proinflammatory cytokines leading to gastrointestinal symptoms.[165,167]

Therefore, given the current uncertainties regarding which gluten-based constituent is triggering symptoms (Figure 8), it is of some investigators' opinion that patients should be informed that owing to the absence of diagnostic biomarkers, their condition can be considered as "self-reported" NCGS or non-coeliac wheat sensitivity, or "patients who avoid wheat and/or gluten".[168,169]

Figure 8: Proposed effects of wheat-based constituents that trigger clinical symptoms in NCGS [170]



4.2.2 The immunopathogenesis of non-coeliac gluten sensitivity

The immunopathophysiology underpinning NCGS is largely uncertain, with discordant data.[171] In one study, the mucosal response to gluten exposure differed between NCGS and CD.[172,173] Whereas gluten triggered only an innate immune response in NCGS (as demonstrated by increased expression of Toll-like receptors), and showed reduced

intestinal permeability with increased expression of tight junction protein claudin-4, it provoked an additional adaptive immune response (increased expression of IFN-γ, IL-6, IL-21, and IL-17) plus increased epithelial permeability in CD.[172,173] However, increased expression of IFN-γ has been shown in NCGS, opening the possibility of an adaptive component;[174] this concept can be supported by the synthesis of antigliadin antibodies (AGA), seen in a proportion of patients with NCGS, and which can be viewed as activation of adaptive immunity. Elsewhere, preliminary studies on NCGS have demonstrated decreased expression of tight junction proteins in both the small bowel and rectosigmoid mucosa, reduced intestinal barrier function, increased small bowel intestinal permeability, proliferation of peripheral blood monocytes, flow cytometric basophil activation in *in vitro* assays, and eosinophil infiltration of the duodenal and colonic mucosa.[154,175,176] However, the specific gluten peptide triggering mucosal events in NCGS is not clear, with one *in vitro* human study showing gliadin not to induce mucosal inflammation or basophil activation as seen in CD.[177] Yet, gliadin exposure in gluten-sensitive HLA-DQ8 transgenic mice induced immune activation in the absence of intestinal atrophy, paralleled with increased acetylcholine release from the myenteric plexus resulting in increased muscle contractility and epithelial hypersecretion, with the abnormalities reversed following gluten withdrawal.[178]

4.2.3 The absence of diagnostic biomarkers for non-coeliac gluten sensitivity

To date no biomarkers for NCGS have been identified, although some investigators report that 25-50% of patients may have serum AGA, mainly IgG class.[154,172,173,179-181] However, AGA lack specificity as they can be present in the general population and healthy blood donors (2-12%), patients with IBS (6-17%), connective tissue disorders (9%), and autoimmune liver

diseases (21.5%).[182] Nevertheless, their presence in the context of NCGS would help support its diagnosis, as a GFD correlates with clinical and serological remission.[181] Duodenal biopsies in NCGS are normal or demonstrate a mild increase in IEL, usually ranging from 25-40 per 100 enterocytes, which is less than that characteristically seen in CD.[172,173,179] Furthermore, the duodenal IEL pattern in NCGS may show a peculiar distribution, with clusters of lymphocytes in the superficial epithelium and linear deposition within the lower portion of the lamina propria.[183,184] However, these findings have also been seen in IBS cases without NCGS, indicating a lack of specificity.[184]

4.2.4 Attempting to differentiate non-coeliac gluten sensitivity from coeliac disease

Despite NCGS seemingly having a relatively simple definition difficulties do arise in adequately excluding CD. Importantly individuals who seek medical attention for gluten sensitivity might already be on a GFD, which in the context of CD can eliminate serological markers and normalize duodenal biopsies.[185] HLA-DQ typing can be useful in that a negative HLA-DQ2 and HLA-DQ8 result excludes CD with certainty.[186] However, if HLA-DQ typing is not readily available, or is positive for HLA-DQ2 and/or HLA-DQ8, then patients might need to reintroduce gluten into their diet prior to formal testing for CD.[186] Understandably, patients might be apprehensive about undertaking a gluten challenge, which historically entailed 10 grams of gluten (~ 4 slices of bread) per day for 6 weeks. However, data have shown that a 2-week challenge of 3 grams of gluten (~1.5 slices of bread) per day might suffice and induces histological and serological abnormalities in the majority of adults with known CD.[187] In this study, the histological abnormalities of villous atrophy were apparent in 68.4% at day-14, whereas serum TTG-2 and deamidated gliadin antibody titres rose from 50% at day-14 to 75% at day-28. Using this combined approach, evidence of serological or

histological abnormalities for CD were detected in 89.5%.[187] Nevertheless, this proposed algorithm is yet to be adopted globally.

Alternatively, in those who are unable to perform an oral gluten challenge, an *in vitro* gliadin challenge of duodenal mucosa can help identify cases of CD, but the availability of this test is limited to selected tertiary-care centres only.[188]

4.2.5 Aims of study

Given that NCGS (or its alternate terminology) appears to be an emerging entity no data exists on the epidemiology and magnitude of this condition within the spectrum of gluten-related disorders. Furthermore, how NCGS differs from CD has not been established.

The aim of this study was to determine the population prevalence of self-reported gluten sensitivity in UK adults and to ascertain the diagnostic yield in those patients referred to secondary gastrointestinal care with gluten-related symptoms. Furthermore, we sought to compare demographic, anthropometric and biochemical differences between patients diagnosed with NCGS against those with CD.

4.3 Methods and Materials

Population Survey

During the period of February to March 2012 a population-based questionnaire was conducted outside large shopping malls and transport stations in Sheffield, United Kingdom (UK). Members of the general public, all over the age of 16 years, participated in the study by filling out a modified version of a previously validated written questionnaire,[102] to which there were three sections (Appendix C). The first comprised basic demographic information

including age, sex and ethnicity. The second section screened for symptoms consistent with irritable bowel syndrome (IBS) in accordance with the Rome III criteria and questionnaire.[15,16] In addition, participants were also asked about their past gastrointestinal, psychiatric and allergic history. The final section of the survey enquired for self-reported gluten sensitivity (group 1) and recognized related symptoms, as demonstrated by recent double-blind placebo-controlled (DBPC) studies and those of expert opinion. Subjects were also asked for their use of a GFD and if they had seen a healthcare professional for their symptoms. A reported diagnosis of CD in the population group was defined by those who had a doctor-diagnosis of CD and were also taking a GFD.

Secondary gastrointestinal care

We also analyzed diagnostic outcomes in all patients referred by General Practitioners to a dedicated secondary-care clinic at the Royal Hallamshire Hospital, Sheffield, UK, between the years 2006 to 2012 (group 2). The referral criteria were "gastrointestinal symptoms attributed to gluten ingestion."

The gold-standard method of delineating true gluten-based sensitivity would be dietary elimination followed by DBPC food re-challenges. However, this is not performed in our clinical practice. Therefore, a pragmatic approach to investigate these patients was adopted. Subjects were openly advised to take a gluten challenge involving ≥3 grams of gluten per day for two weeks prior to undergoing coeliac serology (EMA and TTG) and oesophagogastroduodenoscopy where four distal duodenal biopsies were obtained.[187,189] We do not routinely perform AGA in our clinical practice. IgE-wheat serology was performed to look for wheat allergy.

During the time of initial investigations, body mass index (BMI) was also recorded, as per weight in kg/height in m^2, and classified in accordance with the World Health Organization (WHO) criteria as underweight (<18.5), normal weight (18.5-24.9), overweight (25-29.9) and obese (≥30).[190] Additional laboratory tests included haemoglobin (normal range: females 11.0-14.7 g/dl, males 13.1-16.6 g/dl), ferritin (15-150 ug/L), folate (4.6-18.7 ug/L), vitamin B12 (191-663 ng/L), albumin (35-50 g/L), immunoglobulins, and human leukocyte antigen (HLA) DQ2/DQ8 typing.

The TTG antibodies were assayed by the Sheffield Immunology Department using enzyme-linked immunosorbent assay kits (Aesku Diagnostics, Wendelsheim, Germany). A TTG titer of >15 U/ml was regarded as positive. EMA was detected by immunofluorescence on primate oesophagus sections (Binding Site, Birmingham, UK).

The duodenal biopsies were fixed in formalin at the time of endoscopy. Specimens were then sent to the Sheffield Histopathology Department where they were orientated and embedded in paraffin wax with standard, 3µm thick sections at three levels stained with haematoxylin and eosin. The most severe lesion was noted and graded according to the modified Marsh classification. Although villous atrophy (Marsh 3) is commonly used as the benchmark for diagnosing CD, it is now accepted that CD may present with lesser degrees of histological abnormalities.[191-193] However, there is also evidence to suggest that in cases of NCGS there may be an increase in duodenal IELs (Marsh 1) seen, although the majority of patients will have normal biopsies.[172,173,179]

Consequently, a diagnosis of CD was based on a positive coeliac serology (EMA and/or TTG) and histological abnormalities ranging from Marsh 1 to Marsh 3. A diagnosis of NCGS was

based on negative EMA and TTG, with normal (Marsh 0) to near normal duodenal biopsies (Marsh 1). WA was considered as a diagnosis if IgE-wheat serology was positive.

Comparing NCGS vs. CD

Finally, we retrospectively compared baseline demographic data, BMI, haematological and biochemical parameters for those patients diagnosed with NCGS (in group 2) against a large cohort of patients already under our care with CD based on positive coeliac serology and villous atrophy on duodenal biopsies (n=329; group 3). We did not analyse WA as this is not commonly seen in adult practice.

Statistics and ethical issues

Statistical analysis was carried out by using SPSS version 19.0 software. Categorical variables were summarized by descriptive statistics, including total numbers, percentages, odds ratio (OR), 95% confidence intervals (95% C.I), and associations analyzed by the Chi-Squared test. Continuous variables were summarized by mean and standard deviation (SD), with differences between two groups calculated using the Student T-test for parametric data and Mann-Whitney U-test for non-parametric data. A p-value <0.05 was considered statistically significant.

The study was registered with Sheffield Teaching Hospitals audit and research department and conducted according to principles of the Declaration of Helsinki and Good Clinical Practice Guidelines.

4.4 Results

Population survey

1002 adults completed the population-based questionnaire, with 55% female and 45% male (mean-age 39 years, range 16-93 yrs). Within the general population the prevalence of gastrointestinal conditions was as follows – gastro oesophageal reflux 5%, inflammatory bowel disease (IBD) 1.4%, gastrointestinal cancers (bowel or stomach) 0.5%, and in the absence of any known organic gastrointestinal disorders the prevalence of individuals fulfilling the Rome III criteria for IBS was 6%, of whom 80% were female ($p<0.0001$).

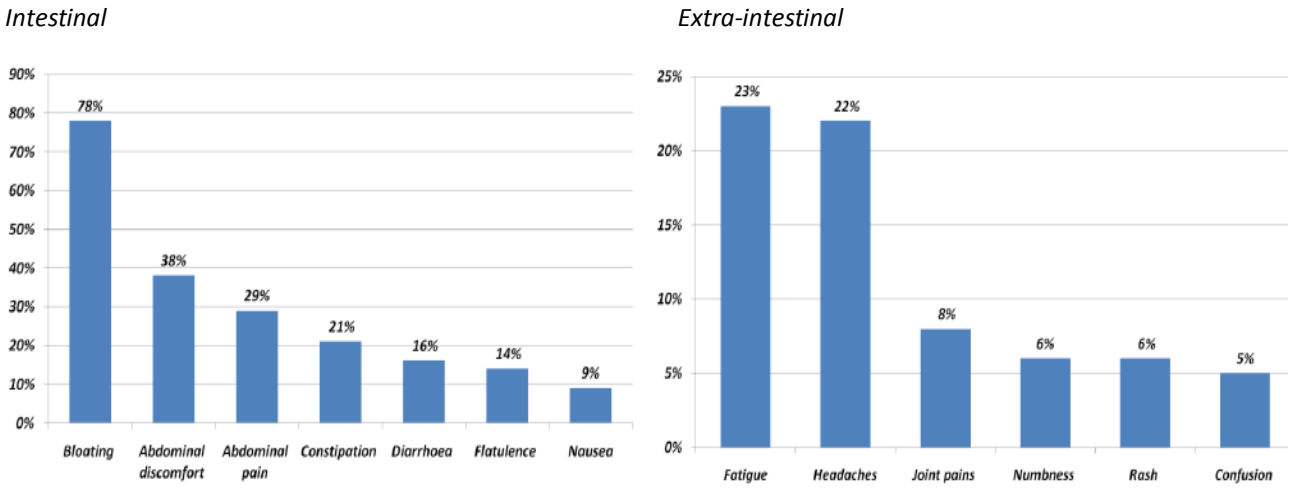
The prevalence of self-reported gluten sensitivity in the general population was 13% (n=129/1002). However, the consumption of a GFD was 3.7% (n=37), with the prevalence of known doctor-diagnosis of CD being 0.8% (n=8). The mean-age of gluten sensitive individuals was 39.5 years, range 18-75 years, and 79% were female, $p<0.0001$ (Table 6). Participants with self-reported gluten sensitivity were more likely to fulfil the Rome III criteria for IBS compared to non-gluten sensitive individuals, with a prevalence of 20% vs. 3.89%, $p<0.0001$, OR 6.23 (CI 3.59-10.8). There was no difference in age, race, gastrointestinal cancers or heartburn between those who were gluten sensitive compared to those who were not gluten sensitive. However, subjects reporting gluten sensitivity had a significantly increased prevalence of anxiety, depression, chronic fatigue syndrome and food allergies/intolerances.

Table 6: Comparison between self-reported gluten-sensitive and non-gluten-sensitive adults in the community (n=1002)

Variable	Gluten sensitive n 129 (13%)	Not gluten sensitive n 873 (87%)	Odds ratio (95% CI)	P-value
Mean age ± SD	39.5 ± 17.7	39 ± 19.7		NS
Sex	79% female : 21% male	51% female : 49% male	3.65 (2.34 – 5.69)	<0.0001
White British	94.6%	94.8%	0.95 (0.42-2.15)	NS
Anxiety	21%	6.8%	3.65 (2.23-6.02)	<0.0001
Depression	13%	6.3%	2.26 (1.27-4.03)	0.0093
Chronic fatigue syndrome	3.1%	0.9%	3.46 (1.03-11.66)	0.0108
Fibromyalgia	0.8%	0.3%	2.27 (0.23-21.9)	NS
Rome III criteria for irritable bowel syndrome	20%	3.89%	6.23 (3.59-10.8)	<0.0001
Gastrointestinal cancers	0.8%	0.46%	1.70 (0.19-15.3)	NS
Heartburn/reflux	8.5%	4.5%	1.99 (0.99 – 4.0)	NS
Nut allergy	3.1%	1.0%	3.07(0.93-10.12)	NS
Egg allergy	3.1%	0.1%	27.9 (3.09-251.67)	0.0012
Dairy intolerance	3.9%	0.9%	4.36 (1.40-13.54)	0.0179

Gluten sensitive individuals described a combination of intestinal and extra-intestinal symptoms in relation to gluten ingestion (Figure 9). The most common intestinal symptoms described were bloating, abdominal discomfort/pain and altered bowel habit (consistent with IBS), with the most frequent extra-intestinal complaints being fatigue and headaches.

Figure 9: Gluten sensitive symptoms reported in the adult community



Secondary gastrointestinal care analysis

200 adults with gluten sensitivity were investigated. The clinic group comprised 84% females, mean-age 39.6 years, range 16-77 yrs, and were therefore similar in age and sex to those complaining of gluten sensitivity in the community.

CD was diagnosed in 7% (n=14 cases) of the gluten sensitive cohort. The remaining 93% (n=186 cases) were classified as NCGS. There were no cases of IgE-wheat allergy. All patients with CD were HLA positive, compared to 53% NCGS cases (p=0.0003).

Differences between NCGS and CD

329 patients with CD were compared to 186 patients with NCGS. The mean-age for patients with CD was 49.8 years in contrast to 39.6 years for patients with NCGS, p<0.0001. Those with NCGS were predominantly female, whereas there was an almost 2:1 female to male ratio in CD, p=0.0013. CD patients were significantly more likely to have co-existing autoimmune disorders, anaemia, low levels of ferritin, folate, vitamin B12 and albumin at baseline (Table 7). There was no statistical difference in family history of CD between the two groups.

Although the mean BMI of CD patients was within the normal range at 23.7, it was significantly lower by roughly two points in comparison to those with NCGS who had a mean BMI of 25.8, p=0.001. Categorizing the BMI of CD and NCGS, respectively, according to the WHO classification showed the following subgroups - underweight (12.7% vs. 5.3%), normal weight (57% vs. 43.7%), overweight (20.6% vs. 29.8%), and obese (9.7% vs. 21.2%).

Table 7: Comparison between CD and self-reported NCGS at baseline diagnosis

Patient characteristics	Coeliac disease (CD) n 329	Non-coeliac gluten sensitivity (NCGS) n 186	Odds ratio (95% C.I)	P - value
Mean age ± SD	49.8 ± 15.7	39.6 ± 15.2		< 0.0001
Sex	71% female : 29% male	84% female : 16% male	0.47 (0.30 - 0.75)	0.0013
Autoimmune history	23.1% (76/329)	9.7% (18/186)	2.80 (1.62 - 4.86)	0.0001
Family history of CD	7.3% (24/329)	12.4% (23/186)	0.56 (0.31 – 1.02)	NS 0.058
Anaemia	25.4% (72/283)	3.3% (6/181)	9.95 (4.23 – 23.4)	< 0.0001
Low Ferritin	48.3% (125/259)	16.2% (23/142)	4.83 (2.90 – 8.03)	< 0.0001
Folate deficiency	29.1% (75/258)	7.2% (11/153)	5.29 (2.71 – 10.34)	< 0.0001
Vitamin B12 deficiency	13.3% (34/256)	3.9% (6/155)	3.80 (1.56 – 9.28)	0.0017
Hypoalbuminaemia	10.2% (29/283)	2.8% (5/178)	3.95 (1.50 - 10.41)	0.003
Mean BMI ± SD	23.7 ± 5.45	25.8 ± 5.59		0.001

Values given in percentages with numerator (patients with altered parameter)/denominator (total checked) stated in brackets.

4.5 Discussion

To our knowledge this is the first study of its kind to assess the prevalence of self-reported gluten sensitivity in the community and analyze diagnostic outcomes in those referred to secondary gastrointestinal care. We have shown that gluten sensitivity is self-reported by 13% of the population, with 3.7% consuming a GFD, despite only 0.8% being aware that they have a formal diagnosis of CD. Gluten sensitive individuals are predominantly female, report an association with IBS, and experience both intestinal and extra-intestinal symptoms on gluten ingestion. Of those patients presenting to the gastroenterology department the majority do not have CD but self-reported NCGS. In contrast to CD, we have established that NCGS patients have a higher mean BMI by two points and are significantly less likely to suffer complications such as nutritional deficiencies and co-existing autoimmune disorders.

Following on from this study several population-based observational studies have also confirmed the avoidance of gluten-based products outside a diagnosis of CD (Appendix A1).[194-200] The reasons for adopting such a lifestyle can be due to a variety of reasons.[198] In some it may be perceived as a healthier option and a means of controlling weight gain by reducing calorific intake; this has been suggested to account for one-in-five cases.[198] However, the majority of individuals taking a self-prescribed GFD do so with the view that gluten exposure triggers symptoms of ill-health.[198]

Previous reports on food intolerance are supportive of my findings. Perceived food hypersensitivity is common, particularly in women, with a reported prevalence of 20.4% in the UK community.[41] A wide range of systemic symptoms may be experienced related to consumption of the intolerant food.[41] In addition, patients demonstrate considerably more generalized subjective health complaints in comparison to healthy controls.[46] A recent case-control study has noted systemic complaints of IBS, musculoskeletal pains and fatigue to be extremely prevalent co-morbidities in those who report food hypersensitivity.[47] Similar results were seen within our gluten sensitive cohort and have been corroborated by other investigators.[126,179,180] Firstly, studies evaluating such patients have noted that NCGS is predominantly an adult condition although paediatric cases have been reported.[201] A prospective multicentre Italian survey performed over 1 year identified 391 new cases of NCGS to 340 new cases of CD, giving a ratio of 1.15:1.[180] In this cohort, the breakdown for adults was 380 NCGS to 302 CD cases (ratio of 1.25:1), whereas for children there were 11 NCGS to 38 CD cases (ratio of 0.29:1).[180] Furthermore, the characteristic phenotype of patients with suspected NCGS is usually young to middle-aged women describing a constellation of both intestinal and extra-intestinal symptoms following gluten exposure

(Appendix B1).[126,179,180,196] In most patients, the time between gluten ingestion and the appearance of symptoms varies from a few hours to 1 day.[180] The most frequent associated disorders are IBS (48%), other food intolerances (35%), which in most cases are represented by lactose intolerance, and IgE-mediated allergy (22%) to inhalants, food, or metals.[180]

Anxiety and depression have also been noted to be a common feature amongst patients with food hypersensitivity.[202] In the context of gluten sensitivity an initial study, which in contrast to those on food hypersensitivity, interestingly suggested there to be a low level of somatisation and no elevation in anxiety and depression. However, this study was restricted in patient number and all were on a GFD at the time of baseline questionnaires which may possibly explain the negative findings.[203] This suggestion would be supported by studies in other patient groups where for example cure of duodenal ulcers leads to normalization of anxiety and neuroticism.[204] In fact, re-introduction of gluten has been shown to cause depression in NCGS, as seen in a small DBPC crossover trial where 22 patients with NCGS randomly received one of three dietary challenges (gluten, whey, or placebo) for 3 days, followed by a minimum 3-day washout before crossing over to the next diet. The mental state at the end of each challenge was assessed using the Spielberger State-Trait Personality Inventory, a validated tool measuring anxiety, depression, anger and curiosity. Short-term gluten exposure specifically induced current feelings of depression with no effect on other indices or on emotional disposition.[205]

It was also apparent that many subjects reported neurological manifestations following gluten exposure. Indeed, there is a growing body of literature to show that even in the absence of CD gluten causes neurological manifestations in the form of ataxia, neuropathy and encephalopathy.[206,207] Gluten ataxia is the most common neurological disorder to have

been studied.[207] In a case series of 1,000 patients with progressive ataxia, 18% of patients had positive AGA; amongst patients with idiopathic sporadic ataxia the prevalence of AGA was 43% compared with 12% in a healthy population and 13% in patients with genetically characterized ataxia.[207] Most patients with gluten ataxia did not have gastrointestinal symptoms, and 60% demonstrated normal histology on duodenal biopsy samples.[207] 60% of patients with gluten ataxia had evidence of cerebellar atrophy on magnetic resonance imaging (MRI) and all patients had abnormal MR spectroscopy of the cerebellar vermis suggesting abnormal cerebellar neuronal physiology independent of atrophy (Appendix C1).[207] Post-mortem examination of patients with gluten ataxia showed patchy loss of Purkinje cells throughout the cerebellar cortex, but also evidence of inflammation with perivascular lymphocytic cuffing. The clinical response to a GFD depends on the duration of ataxia as prolonged gluten exposure results in irreversible loss of Purkinje cells with atrophy of the cerebellum (Appendix C1).[208]

Similar observations have been made in gluten-induced peripheral neuropathy.[207] In one study, 34% of patients with idiopathic sporadic neuropathy had circulating AGA, of which 74% did not have any evidence of enteropathy.[209] In those on a GFD, circulating AGA were eliminated and neuropathy substantially improved compared with those who maintained gluten consumption;[210] improvement was seen irrespective of enteropathy.

Gluten encephalopathy refers to a combination of intractable headaches often with abnormal brain white matter on MRI. The headache improves after the introduction of a GFD. Possible cognitive deficits associated with such MRI findings remain to be explored. In this group of patients, 43% do not have enteropathy, yet a GFD arrests progression of white matter abnormalities on MRI (Appendix D1).[207,211]

Skin rash was also described by many self-reporting gluten sensitive individuals. Although dermatitis and eczema are frequently reported after gluten exposure, only the relationship between psoriasis and NCGS has been further evaluated. Psoriasis in patients who are AGA positive can be improved by a GFD.[212] The investigators performed a case–control study involving 33 patients with psoriasis who were AGA positive and six AGA-negative patients. 31 AGA-positive patients were negative for serum EMA, with duodenal biopsies showing either increased duodenal IELs or normal biopsy. After a 3-month period on a GFD, the AGA-positive cohort showed a notable improvement in the psoriasis area and severity index as well as reduction in AGA values. This improvement was not seen in the AGA-negative cohort. When a gluten-containing diet was recommenced there was a deterioration of psoriasis in just over one-half of the AGA-positive patients.[212]

A substantial proportion of patients reported gluten to also induce musculoskeletal pains and fatigue, symptom manifestations that are akin to fibromyalgia. In fact, A case series has shed light on the potential benefits of a GFD in patients with fibromyalgia.[213] In 20 patients with longstanding and relatively debilitating symptom history of fibromyalgia a GFD was trialled after conventional therapies failed. CD was excluded by negative TTG-2 antibodies and absence of villous atrophy, although all patients were noted to have increased duodenal IELs. After commencing a GFD, clinical response led to at least one of the following scenarios: remission of fibromyalgia pain criteria; return to work; return to normal life; or discontinuation of opiates. The reintroduction of gluten was followed by fibromyalgia relapse, which subsided upon a GFD.[213] Following on, a case–control study evaluated the effects of a GFD in 97 patients with fibromyalgia and coexisting IBS, in which 58 patients had raised duodenal IEL and 39 had normal duodenal biopsies.[214] Coeliac serology was negative.

At baseline, all participants recorded similar poor quality of life and high fibromyalgia and IBS-related symptom scores. After 1 year on a GFD, all outcome measures markedly improved by 26-30% in the increased duodenal IEL group compared with 3-4% in the normal mucosa group. These results stress the potential role of gluten as a trigger of the clinical manifestations of IBS and fibromyalgia and indicate that increased duodenal IEL might be a useful clue to identify those patients who potentially benefit from gluten withdrawal.[214]

In secondary gastrointestinal care, we identified that 93% of patients investigated for self reported gluten sensitivity had NCGS, with the remaining 7% fulfilling the criteria for CD. Various other groups have also undertaken this evaluation and shown the prevalence of CD to range from 2% to 42.7% (Appendix E1).[186,196,215-218] The discrepancy in results may be explained by the current lack of international consensus with regards to the optimal dosage and duration of gluten-challenge as well as the criteria used to make a diagnosis of CD.[217] Nevertheless, similar to reports from other investigators, all patients with CD were HLA-DQ2 and/or DQ8 positive in comparison to 53% with NCGS.[173,179] Finally, we have established clinical differences between CD and NCGS. Previous studies have shown CD to be commonly diagnosed between the fourth to six decades, with a 2:1 female to male preponderance.[219,220] Our findings show similar results with regards to CD patients, but also demonstrate that in comparison NCGS patients are predominantly women who on average tend to be ten years younger at the time of diagnosis. This difference may be explained by the specific presentation of NCGS patients, who on reporting gluten related symptoms are directly investigated for associated disorders, thus potentially allowing a prompt diagnosis. However, in CD the presentation can be non-specific and heterogeneous, which may lead to multiple investigations, incorrect initial diagnoses (i.e. IBS), and in some cases consultations

with numerous physicians including gastroenterologists before the eventual diagnosis of CD is reached. As a result, reports suggest that there may be a delay in diagnosis of CD by around a decade.[220-223]

We have shown that CD patients are significantly more likely to have nutritional deficiencies, anaemia and a lower mean BMI compared to those with NCGS. These findings are consistent with previous reports on CD and biologically plausible due to the reduced absorptive capacity of the proximal small bowel secondary to villous atrophy.[224-227] There was also a significantly greater association of autoimmune disorders in patients with CD compared to NCGS (23.1% vs. 9.7%). The autoimmune disorders noted in the NCGS group were 14 cases of thyroid disease and one case each of Sjogren's syndrome, type 1 diabetes mellitus, autoimmune thrombocytopenic purpura and rheumatoid arthritis. Previous studies have shown that roughly 30% of adults with CD may have one or more autoimmune disorders.[228-230] It has been postulated that dysfunction in T-regulatory cells accounts for the loss of immune homeostasis and the development of autoimmunity as seen in CD and related conditions.[231] On reflection, our study would have benefited from having a healthy control group although elsewhere investigators have now shown that NCGS subjects are generally more likely to have nutritional deficiencies, coexisting autoimmunity, a lower mean BMI and decreased bone mineral density than the general population; however, these complications are seen less frequently in NCGS than in CD, supporting our findings. [169,179,232]

Both CD and NCGS patients share a similar prevalence of an affected first degree relative with gluten sensitive enteropathy (7.3% vs. 12.4%, respectively). The prevalence of a family history of CD can be seen in 5-24% of patients with NCGS.[154,169,179,180,186,196,232] This finding might reflect that individuals who self-prescribe a GFD do so because they are aware of CD,

and its protean manifestations, through their family history. However, up to half of non-coeliac siblings of patients with CD demonstrated gluten sensitivity following a rectal gluten challenge,[233] suggesting that within a family various degrees of gluten sensitization exist which requires further exploration.

This study has limitations. Firstly, the population-based study was a self-completed questionnaire for which we were not able to investigate further. Nevertheless, the individuals self-reporting gluten sensitivity in the population appear to have similar characteristics to those referred to secondary-care. Secondly, there can be a discrepancy between perceived food intolerance and the gold standard method of testing which is dietary elimination followed by DBPC food re-challenges.[41] This may, therefore, have led to an over representation in our samples as it has recently been shown that of those complaining of gluten sensitivity 30% of patients are truly sensitive to gluten-based products.[154] Finally, there is uncertainty as to whether it is gluten withdrawal which benefits patients or another component of wheat such as FODMAPs or non-gluten proteins.

4.6 Conclusion

Sensitivity to gluten based products is a common complaint self-reported by the population, with the use of a GFD outweighing the prevalence of known CD. Affected subjects report a wide range of symptoms related to gluten ingestion and there is a relationship with IBS. Of those investigated by gastroenterology in secondary-care, 7% have CD and 93% could be termed as NCGS. Subjects with NCGS are unlikely to present with the clinical complications associated with CD.

CHAPTER 5: Evaluating the bidirectional relationship between inflammatory bowel disease and self-reported non-coeliac gluten sensitivity

5.1 Summary

Background & Aims: Non-coeliac gluten sensitivity and the associated use of a gluten-free diet (GFD) are perceived to belong to the spectrum of irritable bowel syndrome (IBS). However, recent reports also suggest substantial use of a GFD in inflammatory bowel disease (IBD). We assessed the bidirectional relationship between IBD and self-reported non-coeliac gluten sensitivity (SR-NCGS). **Methods:** A cross-sectional questionnaire screened for SR-NCGS, and the use of a GFD, in four groups; Ulcerative colitis (n=75), Crohn's disease (n=70), IBS (n=59) and dyspeptic controls (n=109). We also assessed diagnostic outcomes for IBD in 200 patients presenting with SR-NCGS. **Results:** The prevalence of SR-NCGS was 42.4% (n=25/59) for IBS, followed by 27.6% (n=40/145) for IBD, and least amongst dyspeptic controls at 17.4% (n=19/109); p=0.015. The current use of a GFD was 11.9% (n=7/59) for IBS, 6.2% (n=9/145) for IBD, and 0.9% (1/109) for dyspeptic controls; p=0.02. No differences were established between ulcerative colitis and Crohn's disease. However, Crohn's disease patients with SR-NCGS were significantly more likely to have stricturing disease (40.9% vs. 18.9%, p=0.046), and higher mean CDAI score (228.1 vs. 133.3, p=0.002), than those without SR-NCGS. Analysis of 200 cases presenting with SR-NCGS suggested that 98.5% (n=197) could be dietary-related IBS. However, 1.5% (n=3) were found to have IBD; such patients had associated alarm symptoms, and/or abnormal blood parameters, prompting colonic investigations. **Conclusion:** SR-NCGS is not exclusive to IBS but is also associated with IBD, where its presence may be reflecting severe or stricturing disease. Randomised studies are required to further delineate the nature of this relationship and clarify whether a GFD is a valuable dietetic intervention in selected IBD patients.

5.2 Introduction

Observational studies have demonstrated that self-reported non-coeliac gluten sensitivity (SR-NCGS) and the use of a gluten-free diet (GFD) outweigh the prevalence of coeliac disease (CD) and IgE-wheat allergy. Patients with SR-NCGS experience a constellation of both intestinal and extra-intestinal complaints following gluten exposure. Due to the lack of diagnostic biomarkers and symptom complex, NCGS has been perceived to belong to the spectrum of irritable bowel syndrome (IBS).[132]

However, recent reports have shown that there is also substantial use of a GFD among patients with inflammatory bowel disease (IBD), of whom the majority describe an improvement in their gastrointestinal symptoms and disease course.[234] As these individuals had a low prevalence of CD they could potentially be termed as having SR-NCGS. However, these novel findings raise further questions as to the clinical phenotype of IBD patients with SR-NCGS, and the potential pathogenic mechanisms underlying this relationship. Furthermore, it remains to be elucidated what proportion of individuals presenting with SR-NCGS may actually be harbouring an organic pathology, such as IBD, who would otherwise erroneously be diagnosed as having an IBS-like entity.

In light of this, the aim of this study was to evaluate the prevalence of SR-NCGS in a cohort of patients with IBD. Conversely, we sought to determine the prevalence of IBD in individuals presenting with SR-NCGS.

5.3 Methods and Materials

Assessment of SR-NCGS in patients with IBD

During the period from February to March 2012 a modified version of a previously validated cross-sectional questionnaire[102] was conducted amongst adult patients with IBD (either Crohn's disease or ulcerative Colitis [UC]) attending for their routine out-patient clinic appointment at the Royal Hallamshire Hospital, Sheffield, United Kingdom. In addition, the questionnaire was also completed by two control groups; a cohort with IBS (as defined by the Rome III criteria), and a group of dyspeptic subjects attending open-access endoscopy.

The questionnaire collected information on basic demographic data, whilst also enquiring for sensitivity to gluten-based products and related symptoms (Appendix C). Subjects were also asked if they had a known diagnosis of CD, had ever tried a GFD and whether this diet was currently in place. We classified individuals to have SR-NCGS if they self-reported gluten sensitivity without a known diagnosis of CD. The current gold-standard method of delineating true-NCGS by dietary elimination followed by DBPC food re-challenges is not performed in our clinical practice.

Finally, in those with IBD, an assessment of current disease extent and activity was made by reviewing the clinical notes plus completing validated questionnaires. For subjects with Crohn's disease, this entailed the Crohn's Disease Activity Index (CDAI) score, with remission defined as CDAI score <150, mildly active if 150-219, moderately active if 220-450, and severely active if >450 (Appendix D).[235] For those with UC, the Severe Colitis Activity Index (SCAI) score was used, with a score of <5 defining remission (Appendix E).[236,237]

Assessment of IBD in patients presenting with SR-NCGS

We analysed diagnostic outcomes in all patients referred to our secondary care clinic at the Royal Hallamshire Hospital, between the years 2006 to 2013, with self-reported gluten sensitivity, who following investigations did not have any evidence of CD or IgE-wheat allergy. We evaluated what proportion of these individuals were subsequently found to have IBD and if there were any diagnostic clues in their clinical history.

Statistics

Statistical analysis was carried out using SPSS version 19.0 software. Categorical variables were summarized by descriptive statistics, including total numbers and percentages, and compared between groups using a Pearson Chi-squared test. Continuous variables were summarized by mean and standard deviation (SD), and comparisons between two groups were performed using Student T-test for parametric data and Mann-Whitney U test for non-parametric data. The one-way analysis of variance test was used to compare parametric data across multiple groups. Where appropriate, logistic regression analysis was performed to adjust for age and sex. Statistical significance was set at a p-value of <0.05.

Ethics

The study was registered with Sheffield Teaching Hospitals Research Department and carried out according to the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines.

5.4 Results

Prevalence of SR-NCGS in patients with IBD

A total of 313 individuals completed the questionnaire, the breakdown of which included 145 patients with IBD (75 with Crohn's disease and 70 with UC), 59 with IBS, and 109 dyspeptic controls; Table 8.

No subjects were known to have CD. The prevalence of SR-NCGS was greatest amongst those with IBS at 42.4% (n=25/59), followed by IBD at 27.6% (n=40/145), and least amongst dyspeptic controls at 17.4% (n=19/109); p-value=0.015.

Furthermore, the use of a GFD was similar amongst IBS and IBD, yet significantly greater than that seen in dyspeptic controls. Of the IBS cohort, 15.3% (n=9/59) stated that they had tried a GFD compared to 13.1% (n=19/145) of IBD patients and 1.8% (n=2/109) of dyspeptic controls; p=0.005. The current use of a GFD in cases of IBS was 11.9% (n=7/59), for IBD was 6.2% (n=9/145), compared to 0.9% (n=1/109) for dyspepsia; p=0.02.

Table 8: Comparison between dietary intolerances in IBS, IBD and dyspeptic controls

	IBS (n=59)	IBD (n=145)	Dyspepsia (n=109)	p value*
Mean age (SD)	32.7 (16.1)	45.2 (17.8)	51.7 (20.5)	<0.001
Female (%)	47 (79.7)	91 (62.8)	67 (61.5)	0.04
Nut allergy (%)	2 (3.4)	1 (0.7)	2 (1.8)	0.76
Egg Allergy (%)	3 (5.1)	3 (2.1)	0 (0)	0.05
Dairy intolerance (%)	4 (6.8)	7 (4.8)	2(1.9)	0.2
Self-reported gluten sensitivity (%)	25 (42.4)	40 (27.6)	19 (17.4)	0.015
Ever tried a GFD (%)	9 (15.3)	19 (13.1)	2 (1.8)	0.005
Still on a GFD (%)	7 (11.9)	9 (6.2)	1 (0.9)	0.02

*adjusted for age and sex.

Subgroup analysis of the IBD cohort revealed no statistical differences between patients with Crohn's disease and UC. The prevalence of SR-NCGS in Crohn's disease was 29.3% (n=22/75) compared to 25.7% (n=18/70) in UC; p=0.63. Furthermore, 14.7% (n=11/75) of Crohn's disease patients had tried a GFD compared to 11.4% (n=8/70) UC patients; p=0.56. Finally, the current use of a GFD was also similar between the two IBD groups, at 6.7% (n=5/75) for Crohn's disease and 5.7% (n=4/70) for UC; p=0.81.

Symptoms related to gluten ingestion

Both IBD and IBS patients with SR-NCGS described a constellation of intestinal and extra-intestinal symptoms following gluten exposure; Table 9. In particular, abdominal pain, discomfort, bloating, diarrhoea, fatigue, and headaches were frequent complaints amongst the groups. In addition, both IBS and Crohn's disease subjects reported a similarly high prevalence of flatulence, belching, and nausea following gluten ingestion, compared to UC. Finally, constipation was a predominant symptom in IBS, whereas joint pains were frequently reported in Crohn's disease.

Table 9: Symptoms reported upon gluten exposure in IBS and IBD patients with self-reported NCGS

	Gluten sensitive IBS (n=25)	Gluten sensitive Crohn's disease (n=22)	Gluten sensitive UC (n=18)	p value
Abdominal pain (%)	12 (48)	9 (40.9)	6 (33.3)	0.63
Abdominal discomfort (%)	12 (48)	12 (54.5)	9 (50)	0.9
Bloating (%)	17 (68)	12 (54.5)	14 (77.8)	0.29
Diarrhoea (%)	9 (36)	11 (50)	5 (27.8)	0.34
Constipation (%)	10 (40)	2 (9.1)	2 (11.1)	0.02
Flatulence (%)	8 (32)	11 (50)	2 (11.1)	0.03
Belching (%)	8 (32)	4 (18.2)	1 (5.5)	0.1
Nausea (%)	7 (28)	9 (40.9)	1 (5.6)	0.04
Fatigue (%)	9 (36)	11 (50)	7 (38.9)	0.60
Headaches (%)	11 (44)	6 (27.3)	4 (22.2)	0.27
Joint pains (%)	3 (12)	11 (50)	2 (11.1)	0.003

Characteristics of IBD patients with SR-NCGS

The characteristics of Crohn's disease patients with SR-NCGS compared to those without SR-NCGS are demonstrated in Table 10. There was no difference in demographics or disease location between the groups. However, Crohn's subjects with SR-NCGS were significantly more likely to have stricturing disease (40.9% vs. 18.9%, p=0.046) and a higher mean CDAI score (228.1 vs. 133.3, p=0.002) compared to those without SR-NCGS. Whereas moderate to severe CDAI score was significantly associated with SR-NCGS, a score of <150 was significantly associated with those not reporting gluten sensitivity (p=0.001).

Table 10: Characteristics of Crohn's disease patients with and without self-reported NCGS

	Crohn's disease with SR-NCGS (n=22)	Crohn's disease without SR-NCGS (n=53)	P value
Demographics			
Mean age (SD)	46.6 (19)	47.3 (16.4)	0.87
Female (%)	14 (63.6)	30 (56.6)	0.57
Crohn's disease extent			
Upper gastrointestinal (%)	0 (0)	2 (3.8)	0.36
Small Bowel (%)	13 (59.1)	29 (54.7)	0.73
Ileo-caecal (%)	8 (36.4)	15 (28.3)	0.49
Colonic (%)	9 (40.9)	26 (49.1)	0.52
Crohn's disease severity			
Penetrating disease (%)	0 (0)	4 (7.5)	0.19
Stricturing disease (%)	9 (40.9)	10 (18.9)	0.046
Mean CDAI score (SD)	228.1 (128)	133.3 (104.7)	0.002
Remission: CDAI <150 (%)	7/22 (31.8)	38/52 (73.1)	0.001
Mildly active: CDAI 150-219 (%)	2/22 (9.1)	7/52 (13.5)	
Moderately active: CDAI 220-450 (%)	11/22 (50)	6/52 (11.5)	
Severely active: CDAI >450(%)	2/22 (9.1)	1/52 (1.9)	

In contrast, evaluating the characteristics of UC patients with and without SR-NCGS did not identify any differences according to disease extent or SCAI score. However, UC patients with SR-NCGS were more likely to be women; Table 11.

Table 11: Characteristics of UC patients with and without self-reported NCGS

	UC with SR-NCGS (n=18)	UC without SR-NCGS (n=52)	P value
Demographics			
Mean Age (SD)	38 (15)	45.1 (19.3)	0.16
Female (%)	16 (88.9)	31 (59.6)	0.02
Ulcerative colitis disease extent			
Proctitis (%)	5 (27.8)	16 (30.8)	
Proctosigmoiditis (%)	6 (33.3)	12 (23.1)	
Left sided colitis (%)	2 (11.1)	6 (11.5)	0.67
Extensive colitis (%)	0 (0)	5 (9.6)	
Pancolitis (%)	5 (27.8)	13 (25)	
Ulcerative colitis severity			
SCAI score (SD)	3.56 (2.38)	3.44 (2.53)	0.87

Prevalence of IBD in patients presenting with SR-NCGS

Analysis of 200 cases presenting with SR-NCGS (mean-age 39.1 years, 83% female) identified that 98.5% (n=197) were categorised into the spectrum of dietary related-IBS. However, 1.5% (n=3) of SR-NCGS cases were found to have IBD as part of their initial work-up for gluten sensitivity. These cases are illustrated in Table 12 and demonstrate that such patients presented with additional alarm symptoms, and/or abnormal blood parameters, that prompted colonic investigations other than simply excluding CD and IgE-wheat allergy. The organic diagnoses reached in these three cases include two cases of Crohn's disease, and one case of UC.

Table 12: Cases of IBD identified in patients presenting with self-reported NCGS

Patient demographics	Clinical presentation	Baseline bloods	Diagnosis
25 year old male	6 month history of diarrhoea. Better without gluten-based products.	Raised Erythrocyte sedimentation rate, C-reactive protein, and platelets.	Crohn's pancolitis
39 year old female	Diarrhoea, bloating, abdominal discomfort. Better with GFD.	Raised Erythrocyte sedimentation rate and platelets	Small bowel Crohn's disease
35 year old female	Longstanding history of abdominal discomfort, bloating and constipation. Recent 8 month history of diarrhoea and fresh per rectal blood. Worse with gluten-based products.	Raised C-reactive protein	Ulcerative proctitis

5.5 Discussion

This study has shown that 27.6% of patients with IBD have SR-NCGS, with up to 6.2% currently consuming a GFD of their own volition. Furthermore, in the context of Crohn's disease, the presence of SR-NCGS is significantly associated with concomitant strictures and increased CDAI score. Finally, we have also demonstrated a converse relationship in that 1.5% of cases presenting with SR-NCGS will actually have IBD. However, this study is limited by the relative low number of IBD patients studied and that the self-reporting of NCGS may be higher than actual NCGS.

Is there a biological plausibility for gluten sensitivity and the use of a GFD in IBD patients? Firstly, with the rising incidence and prevalence figures for IBD, there is growing evidence to suggest that environmental factors, such as diet, play a significant but as yet poorly defined role in the pathophysiology of IBD.[238,239] A systematic review evaluating the association

between pre-illness diet and subsequent development of IBD identified high dietary intake of total fats, polyunsaturated fatty acids, omega-6 fatty acids, and meat to be significant risk factors for disease development.[240] Furthermore, in those with established IBD, the majority of patients believe that diet has ongoing implications in perpetuating the natural course of their disease, aggravating clinical symptoms, and diminishing quality of life.[241,242] In fact, up to 65% of patients with IBD self-report food intolerances, with many adjusting their diet accordingly.[241-243] Our findings are similar to those recently published from the United States, where a cross-sectional study involving 1647 patients with IBD identified that 19.1% had previously tried a GFD, with 8.2% currently using a GFD.[234] Importantly, we also demonstrate that the presence of SR-NCGS in patients with Crohn's disease may be a marker to alert clinicians of underlying severe or stricturing disease. This could be due to the physical properties of gluten-based products as a volume effect (high residue) or alternatively there may be specific immunological mechanism which has not been explored. Therefore, future studies assessing the benefits of a GFD on disease activity are needed as the mechanism of dietary-related gastrointestinal symptoms currently encompasses both theories with antigenic stimuli known to induce inflammation, alter gut microbiota, and cause luminal distention.[238,244,245]

For example, in the setting of NCGS associated with IBS, gluten exposure has been shown to activate the innate immune system, with preliminary studies also demonstrating decreased expression of tight junction proteins in both the small bowel and recto-sigmoid mucosa, alterations in small bowel intestinal permeability, presence of antigliadin antibodies, proliferation of peripheral blood monocytes, enhanced cytokine induction, and induction of basophil activation.[154,172-175,179] The group of diarrhoea-predominant IBS individuals at risk of

such effects to gluten appear to be those carrying the HLA DQ2 and/or DQ8 haplotypes.[175,246] Hence, a potential hypothesis, in need of further elucidation, is that NCGS associated with IBD shares a similar genetic susceptibility profile, and pathogenic reaction to gluten, as seen in IBS-related NCGS.

Nevertheless, it must also be borne in mind that gluten is only one of the complex milieu of nutrients present in wheat, and that immune reactions have been noted to occur with other associated constituents. For example, a proportion of patients with Crohn's disease develop *Saccharomyces Cerevisiae* antibodies (ASCA) against the yeast antigen mannan. These individuals with ASCA positive Crohn's disease demonstrate evidence of a disturbed immune response, increased CDAI score, and clinical symptoms following yeast exposure compared to those with ASCA negative Crohn's disease.[247-249] Therefore, it may be that in our study the association between SR-NCGS in Crohn's disease, with the concomitant high CDAI, is rather due to the presence of ASCA positive antibodies aggravating disease activity following exposure to mannans, and not a specific gluten-related effect. However, this would not explain the similarly high prevalence of SR-NCGS seen in UC. Nonetheless, it would be useful to establish ASCA status in future studies as this is not routinely checked in our current clinical practice.

There is also novel data to suggest that amylase-trypsin inhibitors, which are natural pesticides in wheat, can drive an innate intestinal immune reaction via activation of the toll-like receptor 4 leading to up-regulation of pro-inflammatory cytokines.[162,166] Furthermore, recent interest has also emphasised the role of poorly absorbed fermentable carbohydrates (collectively termed FODMAPs) in inducing gastrointestinal symptoms through gaseous production and osmotic diarrhoea.[57,58] Therefore, it can be envisaged that the relationship

between SR-NCGS and severe/stricturing IBD may rather be a consequence of FODMAPs exacerbating clinical symptoms through luminal distension in an already inflamed and stenotic bowel. In fact, a reduction in FODMAPs in IBD has shown to lead to an improvement in overall abdominal symptoms, abdominal pain, bloating, wind and diarrhoea.[250] To summarise, due to the absence of diagnostic biomarkers, it is complex and unclear whether individuals with IBD who have SR-NCGS are actually sensitive to gluten or other components such as mannans, amylase-trypsin inhibitors, or FODMAPS.

An interesting observation noted in this study is that although IBD patients shared similarities in the symptoms generated through consuming gluten-based products, there were some differences. Patients with Crohn's disease who have SR-NCGS were significantly more likely to complain of joint pains compared to their UC counterparts (50% vs. 11.1%). This may be accounted for by the high CDAI seen in gluten sensitive Crohn's disease which can be associated with arthropathy.[251] Alternatively, in view of the co-existing, albeit non-significant, trend towards a greater prevalence of fatigue (50% vs. 38.9%) and headaches (27.3% vs. 22.2%) there may be a role for the transmural nature of Crohn's disease predisposing to increased intestinal permeability and passage of excess gluten peptides into the systemic circulation; there is growing evidence to support the existence of extra-intestinal manifestations in NCGS.[205-207,213,252] In contrast, UC patients with SR-NCGS, compared to Crohn's disease, had a lower prevalence of flatulence (11.1% vs. 50%) and belching (5.5% vs. 18.2%), which may implicate FODMAPs as it has recently been established that the ability to ferment indigestible carbohydrates is diminished in UC, possibly as a reflection of altered gut microbiota.[253]

Finally, there are other limitations to consider for this study. Of those individuals with IBD who had associated SR-NCGS (n=40) we did not routinely exclude CD, although clinical data was available in 20 patients showing no evidence of CD as defined by negative serology and/or normal duodenal biopsies. We do not believe we are missing cases of CD as in our institution we have historically demonstrated the prevalence of CD in patients with IBD to be low at 0.8% (3/354), comparable to that of controls.[254] This data-set has recently been updated showing a similar prevalence at 0.9% (8/884); *unpublished data*. Additionally, a large multi-centre Italian study has reported 0.5% (9/1711) of consecutively enrolled IBD patients to have co-existing CD.[255] Another limitation is that although we have identified that the spectrum of SR-NCGS can include IBD the majority of patients with SR-NCGS were otherwise diagnosed as having dietary-related IBS. However, we did not systematically investigate all patients with SR-NCGS for IBD, or indeed other organic pathologies. The patients that underwent further investigations were those that either reported alarm symptoms or had abnormal blood parameters. Hence, it is possible that we have underestimated the true prevalence of organic diseases in the setting of SR-NCGS. Furthermore, it has recently been shown in a retrospective study that 30% of patients who avoid gluten-based products have alternate diagnoses, most notably small bowel bacterial overgrowth.[169] Therefore, prospective studies are now needed to establish the true prevalence of alternate pathology in individuals presenting with SR-NCGS.

5.6 Conclusion: SR-NCGS is not exclusive to IBS but is also associated with IBD, where its presence may be reflecting severe or stricturing disease. Randomised studies are required to further delineate the nature of this relationship and clarify whether a GFD is a valuable dietetic intervention in selected IBD patients.

CHAPTER 6: Evaluating the effects of a gluten-free diet in diarrhoea-predominant irritable bowel syndrome

6.1 Summary

Background & Aims: A gluten-containing diet alters bowel-barrier function in diarrhoea-predominant irritable bowel syndrome (D-IBS) patients, particularly those who are HLA-DQ2/8 genotype positive. We assessed the clinical response to a gluten-free diet (GFD) in D-IBS patients previously naïve to the effects of gluten and blinded to HLA-DQ2/8 status.

Methods: 48 D-IBS patients (24 HLA-DQ2/8 positive and 24 HLA-DQ2/8 negative) were recruited to undertake a six-week GFD following dietetic input. Validated questionnaires were self-completed at baseline and week-6. The primary endpoint was mean-change in IBS-severity scoring system (IBS-SSS), with a 50-point reduction conferring clinical benefit. Secondary endpoints were change in hospital anxiety and depression score (HADS), fatigue impact score (FIS), and short-form-36 (SF-36). Those IBS-SSS clinical responders who opted to continue with a GFD thereafter were re-consulted at average 18-months to assess durability of the diet, symptom scores, and anthropometric/biochemical status.

Results: Data from 41 patients (76% women, mean-age 40.4yrs) was available for per-protocol-analysis. Of these, 20 were HLA-DQ2/8 positive and 21 HLA-DQ2/8 negative; baseline characteristics were similar other than worse pain-frequency ($p=0.04$), physical-fatigue (0.02), and vitality (0.05) in the HLA-DQ2/8 positive group. Overall, a six-week GFD reduced IBS-SSS ≥50 points in 71% (n=29). In fact, the mean-total IBS-SSS decreased from 286 to 131 points (change -155, $p<0.001$), which was seen similarly across both HLA-DQ groups. However, HLA-DQ2/8 negative subjects showed a greater reduction in abdominal

distension (p=0.04). There was a marked improvement in HADS, FIS, and SF-36 amongst both groups, although HLA-DQ2/8 positive subjects showed a greater response to depression (p=0.02) and vitality (p=0.03) compared to HLA-DQ2/8 negative subjects. In total, 21 of 29 (72%) IBS-SSS clinical responders planned to continue with a GFD long-term; at 18-month follow-up they were still taking a GFD, maintained symptom improvement and demonstrated similar anthropometric/biochemical status relative to baseline.

Conclusion: A dietitian led GFD provides an effective and sustainable treatment option for the management of D-IBS. The pathophysiological mechanism may differ according to HLA-DQ2/8 status. Clinical trials.gov NCT02528929

6.2 Introduction

As discussed in Chapter 1 irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterised by recurrent abdominal pain, or discomfort, associated with an alteration in bowel habit.[1] IBS is common with a pooled global prevalence of 11.2%, shows a female preponderance, and is mainly seen in those under the age of 50 years.[2-4] Despite being a benign disorder by definition, IBS leads a chronic remitting relapsing course with associated fatigue, depression, anxiety, and diminished quality of life (QOL).[6-8]

Prospective cross-sectional studies suggest that diarrhoea-predominant IBS (D-IBS) accounts for almost a third of all subjects with IBS and, moreover, is the predominant subtype encountered in clinical practice.[256,257] Various medications have been proposed to help alleviate the symptoms of D-IBS, including probiotics, tricyclic antidepressants, antibiotics, serotonin antagonists, mesalazine, and loperamide.[258-266] However, these agents carry potential side-effects, and may not be the desired option for long-term use in a relatively

young patient group. Dietary manipulation has also been suggested, particularly as up to 84% of IBS patients believe that food-items trigger their gastrointestinal symptoms.[45,55,267] Of these, gluten-based products are commonly cited as an offending culprit by roughly one-in-four.[45] Indeed, there is growing evidence to show that individuals are placing themselves on a gluten-free diet (GFD) of their own volition even in the absence of coeliac disease (CD).[194-199] This clinical entity has been termed non-coeliac gluten sensitivity (NCGS) following double-blind placebo-controlled studies demonstrating gluten to induce symptoms of IBS, fatigue, and depression.[126,154,156,159-161,205] However, NCGS is not without its controversies as co-existing non-gluten components, such as FODMAPS (fermentable oligo-, di-, mono-saccharides and polyols), can also induce IBS symptoms through mechanisms of gaseous production and osmotic diarrhoea.[56-59] Nevertheless, amidst this cloud of uncertainty, there also remains a paucity of data on whether a GFD can be empirically recommended to D-IBS patients' previously naive to the effects of gluten-based products.

With this in regard, it has recently been demonstrated that D-IBS patients who are human leukocyte antigen (HLA) DQ2/8 genotype positive have accelerated small bowel transit times compared to those who are HLA-DQ2/8 negative.[268] Furthermore, blinded exposure to a gluten-containing diet in the HLA-DQ2/8 positive group reduced tight-junction proteins, increased small bowel permeability, and led to greater stool frequency compared to HLA-DQ2/8 negative subjects.[175] Indeed, previous groups have attempted to evaluate the clinical benefits of a GFD in D-IBS, yielding promising results in the HLA-DQ2/8 positive cohort, but have been limited by patient selection; many of those recruited with D-IBS had potential CD

as evidenced by the presence of coeliac-specific antibodies and raised duodenal intraepithelial lymphocytes on histology.[246]

Therefore, to address these uncertainties we aimed to evaluate the clinical response to a GFD in a rigorously defined cohort of D-IBS patients blinded to their HLA-DQ status. Clinical assessments were made using validated questionnaires for IBS, mood, fatigue, and QOL. Finally, we assessed whether maintaining a GFD was sustainable, beneficial, and its effect on biochemical/anthropometric status.

6.3 Materials and Methods

Participants and setting

This prospective study was carried out at the Royal Hallamshire Hospital, Sheffield, United Kingdom. The hospital provides secondary-care services to a local population of 500 000 people. Following approval from the National Research Ethics Committee the study was conducted between the time periods of September 2012 to July 2015, and registered with Clinical trials.gov NCT02528929.

The inclusion criteria were consecutive British adults attending a gastroenterology out-patient clinic department who fulfilled the Rome III criteria for D-IBS. We excluded CD as per negative serum endomysial/tissue transglutaminase antibodies and normal duodenal biopsies. Additional exclusion criteria were as follows: individuals referred with self-reported gluten sensitivity; patients already on a GFD; individuals with conditions known to mimic D-IBS, such as idiopathic bile acid diarrhoea, pancreatic insufficiency, microscopic colitis, and inflammatory bowel disease.[18-26]

Study protocol

Patients eligible for the study were given verbal information at the time of their first gastrointestinal follow-up clinic consultation, in addition to a patient information sheet. They were made aware that they have a diagnosis of D-IBS (with no evidence of CD), and that the aim of the study was to evaluate the clinical benefits of a GFD. Those patients agreeing to participate were subsequently referred onto one-of-two senior dietitians who provided uniform information on how to undertake a GFD, whilst also providing a gluten-free information pack and contact details in the event of any queries.

The subjects were given validated questionnaires to self-complete at week-0 (the day before commencing a GFD), and then during the GFD period.[269-272] A six-week duration for a GFD was chosen as at the time of commencing this study the clinical entity of NCGS was in its infancy and so we used the example of CD to gauge our estimate. It has previously been shown that in CD the majority of patients (77%) resolve their clinical symptoms within a month after commencing a GFD.[273] However, a small percentage linger on for longer and it was felt that six weeks was an adequate time to capture most responding patients but also maintain compliance within the study given the potential cost implications of a GFD. Following completion of the six-week GFD period, all patients were followed-up by the dietitians where they returned questionnaires and adherence to the GFD was assessed using a validated scoring system.[274] At that point patients in whom IBS symptoms had improved were asked whether they planned to continue with a GFD for the foreseeable future (yes/no answer). In those who answered “yes” a further re-consultation was initiated on average 18-months later to assess sustainability of the diet, symptom scores, and anthropometric/biochemical status.

Blinding of HLA-DQ2/8 genotype

The HLA-DQ2/8 typing was performed during the initial investigation period using the polymerase chain reaction utilizing sequence-specific primers. Patients were grouped as HLA-DQ2/8 positive or HLA-DQ2/8 negative. Only the gastroenterologists were aware of the HLA-DQ2/8 status for the purpose of sequential patient recruitment. Importantly, both dietitians and patients were blinded, and indeed oblivious, to the fact that HLA-DQ2/8 status was being used as the comparative factor.

Self-completed questionnaires

The IBS symptom severity score (IBS-SSS); Appendix F[269] - is a frequently used assessment in clinical studies where responders rate, over the preceding 10 days, abdominal pain severity, pain frequency, bloating, bowel habit dissatisfaction, and life interferences related to bowel symptoms. The maximum cumulative score available is 500, and subjects can be classified as having no symptoms (<75), to mild (75–175), moderate (175-300), and severe IBS (>300). A reduction of 50 points is considered to confer a clinical improvement. This questionnaire was completed by all patients at weeks 0, 2, 4 and 6. In those who opted to continue with a GFD it was completed again at 18-month mean follow-up.

The Hospital Anxiety and Depression Scale (HADS); Appendix G[270] – is a psychological screening tool to which there are in total 14 items, seven each for depression and anxiety. Each item is rated from 0 (not present) to 3 (maximum), giving a cumulative score for each subscale to range from 0 to 21.[270] A subscale score of ≥11 is used to indicate a clinically significant level of anxiety or depression. This questionnaire was completed by all patients at weeks 0 and 6, and at 18-month mean follow-up in those maintaining GFD.

The Fatigue Impact Scale (FIS); Appendix H[271] – consists of a total of 40 questions enquiring for the impact of fatigue over the preceding month. The questions can be subdivided into three sections; physical functioning (10 items), cognitive functioning (10 items) and psychosocial functioning (20 items). Each item consists of a statement, being rated by the subjects as 0 (no problem) to 4 (extreme problem). A higher score represents greater fatigue severity. This questionnaire was completed by all patients at weeks 0 and 6, and at 18-month mean follow-up in those maintaining GFD.

The Short-form 36 (SF-36) questionnaire; Appendix I[272] – measures general health-related QOL over the last month. There are 36 items which can be divided into eight subsections which include physical functioning, physical role, body pain, general health perceptions, vitality, social functioning, emotional role and mental health. These can then be further aggregated to form a physical component summary and a mental component summary. For each subscale, the raw scores are transformed into a scale of 0 to 100, with 100 representing the best possible health-related QOL. This questionnaire was completed by all at weeks 0 and 6, and at 18-month mean follow-up in those maintaining GFD.

Assessment of GFD adherence

A simple, rapid, reliable and validated tool was used to check GFD adherence (Appendix J).[274] This was completed in all patients at the end of the six-week GFD period, and at 18-month mean follow-up in those maintaining GFD. The final score of the questionnaire is made up of five levels (0 to 4), which from a clinical perspective can be grouped into three levels. Patients scoring 0 or 1 do not follow a strict GFD. Patients scoring 2 follow a GFD but with errors necessitating correction. Finally, patients scoring 3 or 4 follow a strict GFD.[274]

Sample size calculation

The sample size calculation was based on the primary endpoint, which was to detect between-HLA-group differences in the mean-change in IBS-SSS following a six-week GFD. In a previous study from Sheffield, UK, the mean IBS-SSS was roughly 270 with a standard deviation of 60.[275] Based on this information a sample size was calculated using computer software PS Power. To detect a clinically relevant change of 50 points on IBS-SSS, with a power of 80% at the 5% level of statistical significance, we estimated 24 participants in each arm. The secondary endpoints were to assess changes in specific IBS symptoms, HADS, FIS, and SF-36 QOL between the HLA-DQ2/8 positive and negative groups. Other secondary endpoints were whether IBS-SSS clinical responders planned to continue with a GFD and if so whether this was sustainable and its effect on clinical status.

Statistical analysis

Statistical analysis was carried out using SPSS version 21.0 software (SPSS Inc. Chicago, USA), with significance set at a *p*-value of <0.05. Categorical variables at baseline (week 0) were summarized by descriptive statistics, including total numbers and percentages, with between-HLA-group comparisons performed using the chi-squared test. Continuous variables at baseline were summarized by mean-values, standard deviation (SD), with between-HLA-group comparisons made using the unpaired Student T-test for parametric data and Mann-Whitney-U test for non-parametric data.

Following a six-week GFD, overall and within-HLA-group changes for IBS-SSS and its associated subscales were analyzed using a repeated measures one-way analysis of variance

(ANOVA) test with post-hoc Bonferroni analysis. A mixed-design ANOVA was used to compare between-HLA-group changes for IBS-SSS/subscales over the six-week GFD period.

With regards to overall and within-HLA-group changes in FIS, SF-36 QOL and HADS, these were analysed using a paired Student T-test for parametric data and Wilcoxan signed-rank test for non-parametric data; data presented using 95% confidence intervals (95% C.I). In contrast, between-HLA-group comparisons for these indices were made after computing for differences at week-6 relative to baseline followed by applying the unpaired Student T-test or Mann-Whitney-U test as appropriate.

Finally, in those patients opting to continue with a GFD comparisons of their body-mass index, haematinics, and symptom scores at 18-month follow-up relative to baseline were made using chi-squared, paired Student T-test, or Wilcoxan signed-rank test as appropriate.

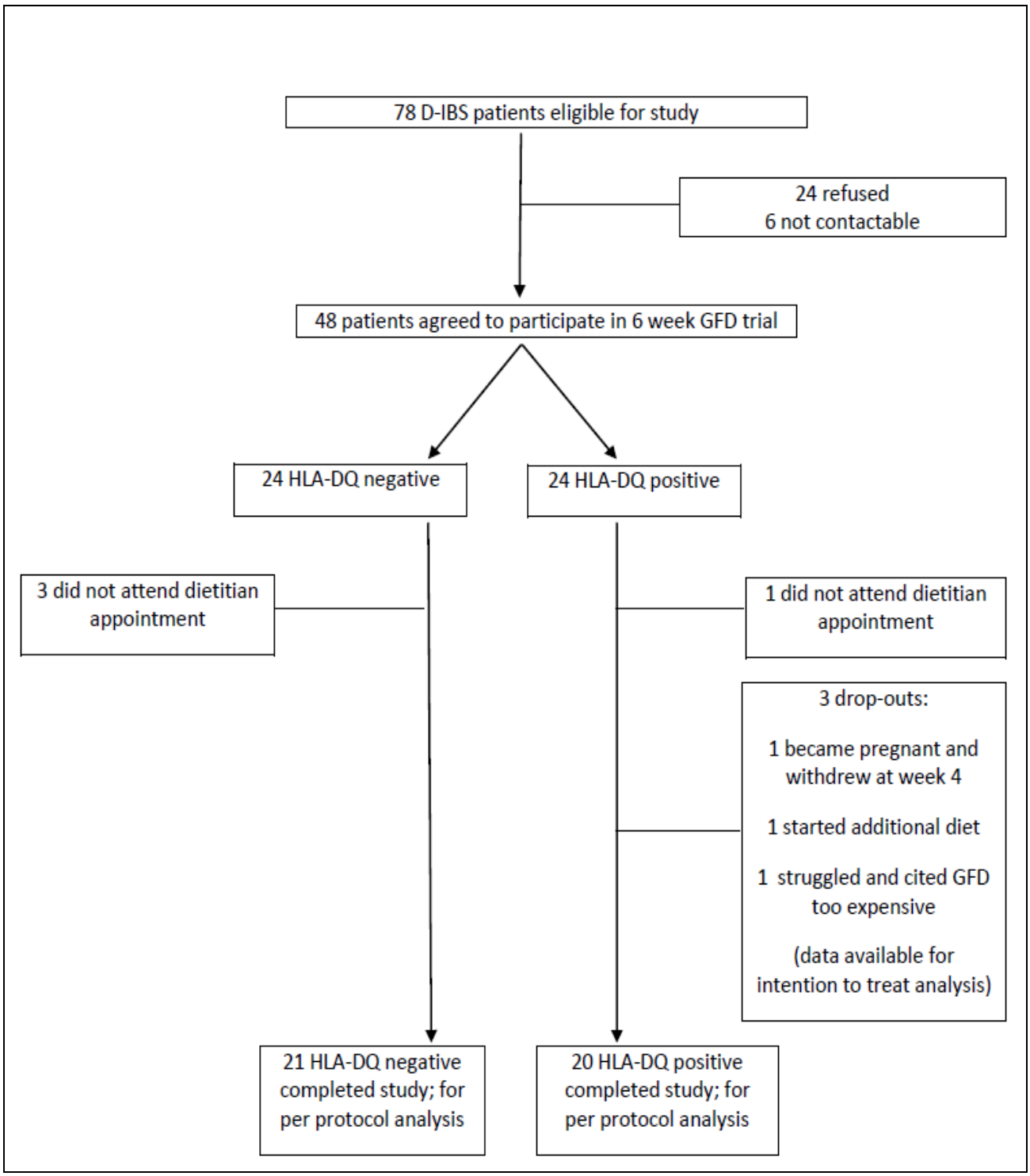
6.4 Results

Recruitment

The required sample size of 48 patients (24 HLA-DQ2/8 positive and 24 HLA-DQ2/8 negative) was reached after sequentially approaching 78 D-IBS subjects eligible for the study; Figure 10. Of the 48 recruited, 1 HLA-DQ2/8 positive and 3 HLA-DQ2/8 negative patients did not attend for their initial dietetic appointment and were excluded from any further analysis. The remaining 44 patients (23 HLA-DQ2/8 positive and 21 HLA-DQ2/8 negative) attended their dietetic appointment and commenced a GFD, although 3 HLA-DQ2/8 positive subjects subsequently dropped out during the study period yet their results were available for intention-to-treat analysis. The per-protocol-analysis was performed in 41

patients (20 HLA-DQ2/8 positive and 21 HLA-DQ2/8 negative) who completed the six-week GFD period and showed a mean GFD adherence score of 3.

Figure 10: Flow chart of D-IBS subject progression for six-week gluten-free diet period



Per-protocol-analysis

Baseline characteristics

The demographic data for the 41 D-IBS patients shows a mean-age of 40.4 years, with 76% female and 95% Caucasian. There was no demographic difference between the HLA-DQ2/8 positive and negative groups (Table 13).

In terms of baseline symptom questionnaire scores, the mean total IBS-SSS was 286.2 which records as being moderate in severity. This was similar between the HLA-DQ groups. Furthermore, there was no difference in the IBS-SSS subscales other than in pain-frequency which recorded to be higher in the HLA-DQ2/8 positive compared to HLA-DQ2/8 negative group (p=0.04).

The HADS, FIS, and SF-36 QOL scores were also similar between the HLA-DQ groups. However, HLA-DQ2/8 positive subjects recorded worse physical-fatigue (p=0.02), total-fatigue (p=0.05) and vitality (p=0.05), with a trend towards a lower SF-36 mental component score (p=0.06); Table 13.

Table 13: Baseline characteristics of D-IBS study participants prior to commencing a GFD

	Overall (n=41)	HLA-DQ2/8 positive (n=20)	HLA-DQ2/8 negative (n=21)	p-value (between HLA-DQ groups)
Demographics				
Mean-age (SD)	40.4 (14.9)	40.9 (16.1)	40.0 (14.1)	0.87
Female (%)	31 (76)	15 (75)	16 (76)	0.93
Caucasian (%)	39 (95)	20 (100)	19 (90.5)	0.5
Employed (%)	30 (73.2%)	13 (65%)	17 (81%)	0.7
IBS-SSS mean-scores (SD)				
Abdominal pain	44 (24)	47.5 (20.9)	40.6 (26.3)	0.33
Pain frequency	59.3 (31.5)	70 (30.6)	49.0 (29.5)	0.04
Abdominal distension	47.9 (26.6)	43.4 (27.8)	52.2 (25.3)	0.29
Stool dissatisfaction	70.9 (23.2)	71.6 (26.2)	70.3 (20.6)	0.77
Life interference	60.4 (22.5)	63.7 (17.7)	57.2 (26.3)	0.36
IBS-SSS total	286.2 (97.7)	299.5 (95.6)	273.6 (100.2)	0.40
HADS mean-scores (SD)				
Anxiety	9.9 (4.8)	10.5 (4.4)	9.4 (9.1)	0.5
Depression	7.0 (4.4)	7.9 (3.8)	6.2 (5.0)	0.24
HADS total	16.9 (8.4)	18.3 (7.2)	15.6 (9.3)	0.31
FIS mean-scores (SD)				
Cognitive	19.2 (13.8)	22.6 (12.5)	16 (14.6)	0.06
Social	15.6 (14.9)	18 (12.9)	13.4 (16.6)	0.13
Physical	18.5 (15.4)	23.4(14.1)	13.7 (15.5)	0.02
FIS total	53.3 (42.1)	64 (40)	43.1 (44.2)	0.05
SF-36 mean-scores (SD)				
Physical function	76 (25.4)	71.5 (25.5)	80.5 (25.1)	0.13
Role physical	50.6 (41)	42.5 (39.8)	58.8 (41.6)	0.21
Bodily pain	50 (25.5)	50.1 (26.4)	50 (25.3)	0.99
General health	50.4 (17.9)	46.9 (18.2)	53.9 (17.9)	0.22
Vitality	39.5 (23.4)	32.3 (19.4)	46.8 (25.4)	0.05
Social function	62.1 (27.4)	62.1 (22)	62 (32.6)	0.7
Role emotional	52.5 (42)	41.7 (40.3)	63.3 (41.8)	0.09
Mental health	53.3 (20.7)	50.9 (17.9)	55.6 (23.4)	0.48
SF-36 PCS	56.4 (20.4)	52.8 (20)	60 (20.8)	0.27
SF-36 MCS	50.7 (23)	44.7 (15.1)	56.7 (27.9)	0.06

Response to a six-week GFD

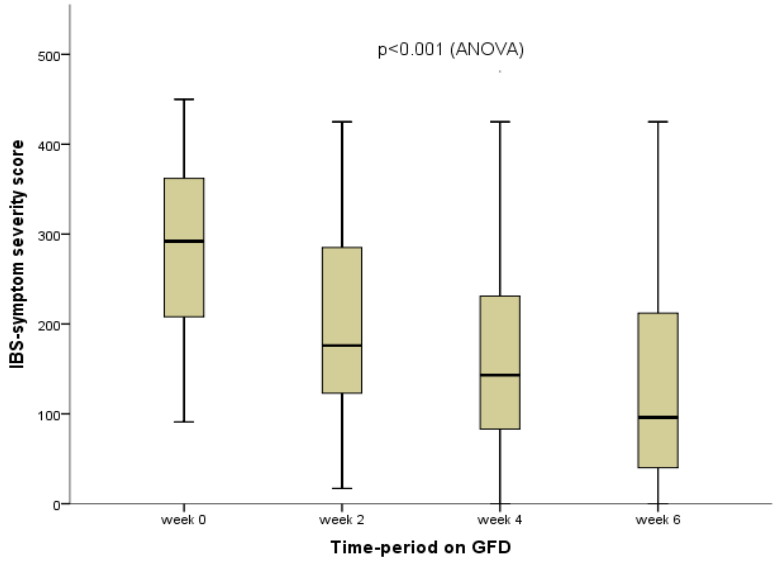
i) IBS severity scoring system

There was a significant overall reduction in IBS-SSS following a six-week GFD (Figure 11). The mean total IBS-SSS score dropped from 286.2 at baseline to 131.5 by week-6 (mean-change, -155 points; 95% C.I, -213.3 to -96.2 points; $p<0.001$). In fact, a significant symptom reduction was seen as early as week-2 (mean-change, -83 points; $p<0.001$) and continued to drop between each interval at week-4 ($p=0.03$) and week-6 ($p=0.03$).

When comparing the response to a GFD according to HLA-DQ subtype, both HLA-DQ2/8 positive and negative subjects showed a significant reduction in the IBS-SSS ($p<0.001$). However, there was no detectable difference between the two groups with the mean-change in the HLA-DQ2/8 positive group being -153 points (95% C.I, -237.6 to -67.7 points) and in the HLA-DQ2/8 negative group being -156.7 points (95% C.I, -248.6 to -91.2 points); Figure 12. In terms of the subscales representative of the IBS-SSS there was a significant mean-reduction in abdominal pain, pain frequency, stool dissatisfaction, and life interferences in both HLA-DQ groups but no differences between the groups over the six-weeks (Figure 12). However, HLA-DQ2/8 negative subjects showed a significantly greater reduction in abdominal distension compared to HLA-DQ2/8 positive subjects ($p=0.04$).

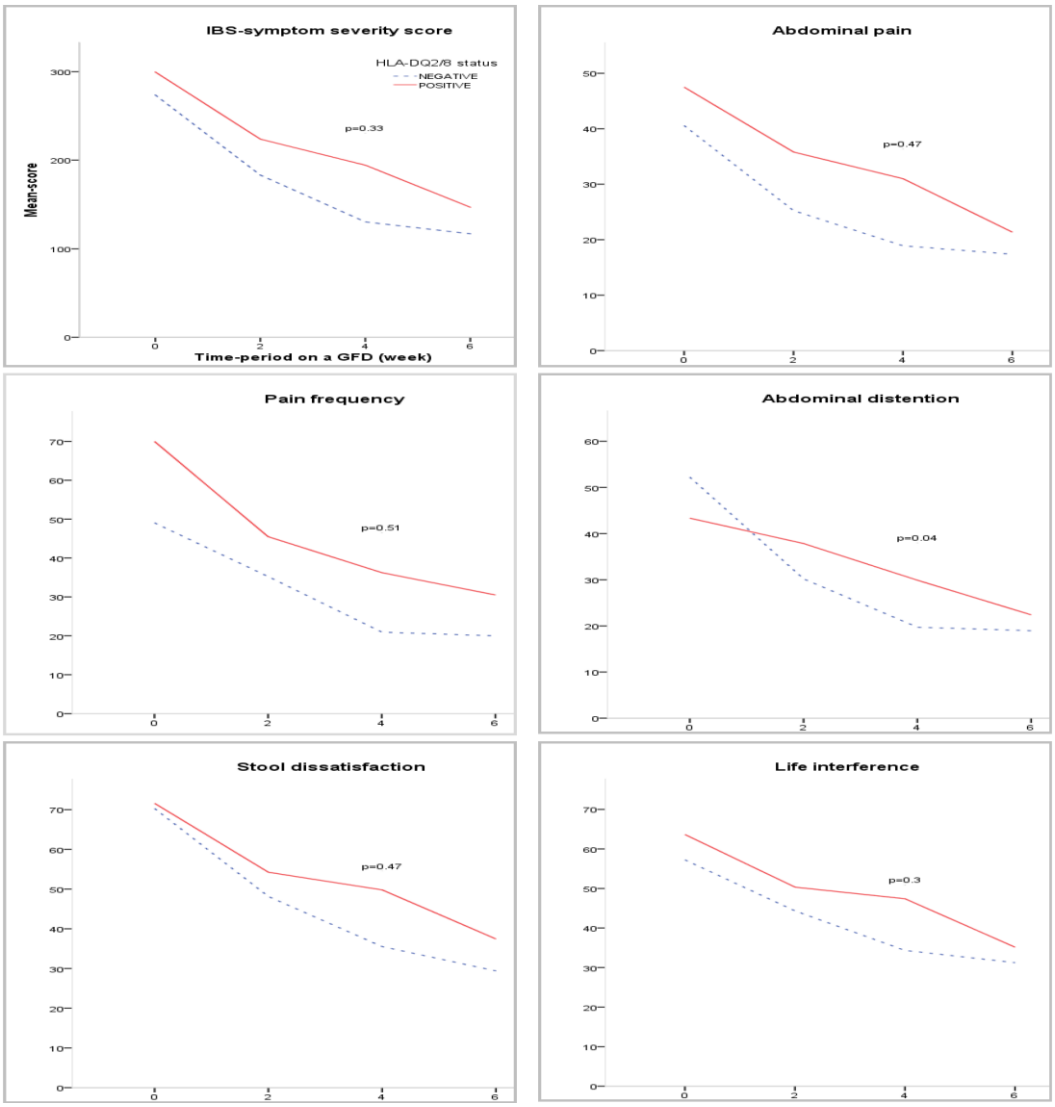
Overall, a reduction of IBS-SSS by ≥50 points, which confers clinical benefit, was seen in 71% (n=29/41) of subjects. This was similar across both HLA-DQ groups, being seen in 70% (n=14/20) of the HLA-DQ2/8 positive and 71.4% (n=15/21) of the HLA-DQ2/8 negative group; p-value=NS.

Figure 11: Change in IBS severity score during a six-week GFD in all subjects



Box plot showing 25/50/75 percentiles. Whiskers represent minimum and maximum values

Figure 12: Change in IBS severity score & subscales during a six-week GFD according to HLA-DQ status



ii) Hospital anxiety and depression scores

There was a significant improvement in HADS following a GFD, which was generally seen in both HLA-DQ groups (Table 14). However, the HLA-DQ2/8 positive group conferred a greater improvement in depression (p=0.02) and total-HADS (p=0.05) compared to the HLA-DQ2/8 negative group.

iii) Fatigue impact scores

There was an overall significant improvement in FIS following a GFD, seen in both HLA-DQ groups (Table 14). However, the HLA-DQ2/8 positive group conferred a trend towards a greater improvement in physical-fatigue (p=0.07) and total-fatigue (p=0.09), compared to the HLA-DQ2/8 negative group.

iv) Short form-36 QOL scores

A GFD led to an overall improvement in SF-36 QOL which was seen across both HLA-DQ groups (Table 14). However, HLA-DQ2/8 positive patients showed a significantly greater improvement in vitality compared to HLA-DQ2/8 negative patients (p=0.03).

Table 14: Mean change in HADS, FIS, and SF-36 QOL from baseline after a six-week GFD in D-IBS subjects (95% C.I)

	Overall (n=41)	HLA-DQ2/8 positive (n=20)	HLA-DQ2/8 negative (n=21)	p value (between HLA-DQ groups)
HADS				
Anxiety	-1.85 (-0.8, -2.9)	-2.5 (-0.6, -4.4)	-1.2 (-0.2, -2.3)	0.27
Depression	-2.1 (-1.0, -3.2)	-3.4 (-1.6, -5.2)	-0.86 (+0.4, -2.1)	0.02
Total	-4.0 (-2.1, -5.8)	-5.9 (-2.6, -9.2)	-2.1 (-0.3, -3.9)	0.05
FIS				
Cognitive	-7.1 (-3.7, -10.5)	-10.3 (-4.1, -16.4)	-4.0 (-0.8, -7.3)	0.10
Social	-5.95 (-2.6.-9.3)	-8.1 (-2.3, -13.9)	-3.9 (-0.2, -7.7)	0.15
Physical	-7.1 (-3.1,-10.6)	-10.9 (-4.3, -17.4)	-3.5 (-0.8, -6.2)	0.07
Total	-20.1 (-10.3, -30)	-29.2 (-11, -47.4)	-11.4 (-3.0, -19.9)	0.09
SF-36 QOL				
Physical function	+7.5 (+2.1, +12.9)	+12.0 (+1.5, +22.5)	+3.0 (+0.1, +5.9)	0.4
Role physical	+32.9 (+19.9, +45.9)	+41.0 (+23, +59)	+24.7 (+5.0, +44.5)	0.2
Bodily pain	+19.1 (+11.3, +26.9)	+16.9 (+4.8, +29)	+21.4 (+10.4, +32.4)	0.6
General health	+7.7 (+2.1, +13.2)	+8.35 (-1.5, +18.2)	+7.0 (+0.95, +13.0)	0.8
Vitality	+14.0 (+6.9, +21.1)	+20.7 (+9.1, +32.3)	+7.25 (+0.91, +15.4)	0.03
Social function	+13.0 (+3.8, +22.2)	+14.3 (-1.6, +30.1)	+11.8 (+0.7, +22.9)	0.8
Role emotional	+16.7 (+3.5, +29.9)	+25.1 (+3.0, +47.1)	+8.3 (-7.6, +24.3)	0.06
Mental health	+9.95 (+3.4, +16.5)	+13.0 (+2.3, +23.7)	+6.9 (-1.4, +15.2)	0.35
SF-36 PCS	+14.4 (+7.7, +21.1)	+14.9 (+4.2, +25.8)	+14.0 (+4.8, +23.2)	0.9
SF-36 MCS	+14.4 (+7.2, +21.5)	+20.3 (+8.0, +32.5)	+8.5 (+0.7, +16.2)	0.1

PCS, Physical component score; MCS, Mental component score

After-care

At the end of the six-week GFD period, 72% (21/29) of IBS-SSS clinical responders planned to continue with a GFD for the foreseeable future; 11 were HLA-DQ positive and 10 HLA-DQ negative (p=NS). These 21 patients were re-consulted on average 18-months later (range 7-26 months) and were still maintaining a GFD with a mean-adherence score of 3, had ongoing symptom score improvement, with no alterations in body-mass index or biochemical status relative to baseline; Table 15. All reported that inadvertent gluten exposure triggered IBS symptoms.

Table 15: Comparison of body-mass index, blood parameters, and symptom scores at mean 18-months compared to baseline in D-IBS patients maintaining a GFD (n=21)

	Pre-GFD	On GFD (mean 18-months)	p-value
Mean anthropometric and biochemical status			
Body-mass index ± SD	27.3 ± 5.4	26.7 ± 5.0	NS
Haemoglobin g/dl (% normal)	14.2 (100%)	13.8 (95%)	NS
Ferritin µg/l (% normal)	77.7 (87.5%)	83. 3 (90%)	NS
Folate µg/l (% normal)	8.1 (100%)	8.8 (95%)	NS
Vitamin B12 ng/l (% normal)	388.6 (100%)	342.8 (95%)	NS
Albumin g/l (% normal)	46.7 (100%)	45.3 (100%)	NS
Mean symptom scores ± SD			
IBS symptom severity	313.5 ± 97	95.5 ± 75	<0.001
Hospital Anxiety and Depression	17.8 ± 8.6	9.35 ± 8	<0.001
Fatigue Impact Scale	58.9 ± 45.7	20.9 ± 33.5	<0.001
Short Form-36 PCS	52.3 ± 19.5	79.9 ± 16.9	<0.001
Short Form-36 MCS	47.4 ± 23.8	76 ± 21.8	<0.001

Intention-to-treat analysis

A total of 44 patients (23 HLA-DQ2/8 positive and 21 HLA-DQ2/8 negative) were available for the intention-to-treat analysis. A GFD led to an overall and within-group improvement in IBS-SSS, FIS, HADS and SF-36 QOL. However, between-group comparisons revealed that a GFD conferred greater benefit in HLA-DQ2/8 positive subjects with regards to depression ($p=0.04$), vitality ($p=0.02$), and role emotional ($p=0.05$) compared to HLA-DQ2/8 negative subjects. In contrast, HLA-DQ2/8 negative subjects experienced greater resolution in abdominal distension ($p=0.03$)

6.5 Discussion

This prospective study demonstrates that 71% of subjects with D-IBS show clinical response to a six-week GFD, with additional improvements in mood, fatigue and QOL also noted. These findings occurred irrespective of HLA-DQ status, although HLA-DQ2/8 positive subjects conferred a greater improvement in depression and vitality compared to their HLA-DQ2/8 negative counterparts. In contrast, HLA-DQ2/8 negative subjects showed a greater reduction in abdominal distension. Finally, 72% of patients planned to continue with a GFD long-term, and on review mean 18-months later were still maintaining the diet, reported ongoing symptom-remission, and did not show any detrimental effects to body-mass index or haematinic status.

The strengths of this study include the methodology used. We recruited a rigorously defined cohort of subjects with D-IBS who had no evidence of organic pathologies. In particular, we ensured exclusion of CD, as demonstrated by negative coeliac serology and normal duodenal biopsies in all patients, to prevent any ambiguity when interpreting the findings.

This contrasts to previous studies that on attempting to evaluate the clinical effects of a GFD in D-IBS have been limited by including those with potential CD, as evidenced by the presence of coeliac related antibodies and/or raised duodenal intraepithelial lymphocytes.[246] Furthermore, we ensured that both patients and dietitians were blinded, and indeed oblivious, to the fact that HLA-DQ2/8 status was being used as the comparative factor. Finally, this was a real life pragmatic study where the onus was left upon the patients to take a GFD following a single dietetic clinic appointment, as opposed to being in a heavily controlled research environment where all meals are provided. Furthermore, we were able to demonstrate safety and durability of the diet with ongoing symptom remission. Hence, we believe that our findings can be generalised and applicable to D-IBS patients when they are seen in clinical practice.

The limitations of this study include the placebo-effect of undertaking a dietary trial. However, systematic meta-analysis of randomised controlled trials in IBS have demonstrated a pooled placebo response rate of 37.5%, with lower responses seen in those who fulfil the Rome criteria on study entry and that used 8 weeks or more of therapy.[276] Indeed, a placebo effect of 33% was found in a recent trial performed on IBS subjects within Sheffield.[275] This would suggest that in our study the 71% response rate to a GFD at six-weeks is unlikely placebo particularly as well-being was maintained at mean 18-months despite having had no interim office visits. Furthermore, other investigators have recently shown a similar response-rate with 80/102 IBS patients improving following a six-week GFD, with subsequent double-blinded exposure to gluten-containing powder leading to significant symptom deterioration in 74.3%, compared to 16.2% of those receiving gluten-free powder.[160] These findings would therefore support the use of a GFD in D-IBS. However,

it is not known how a GFD directly fares in comparison to other dietary therapies that have been proposed to alleviate the symptoms of D-IBS. For example, the low-FODMAP diet has been shown to effectively reduce gastrointestinal symptoms in around 50-70% of IBS patients,[58] although most recent data from a 4-week multicentre study would suggest that simple dietary interventions (such as regular meal patterns; avoidance of large meals; and reduced intake of fat, insoluble fibers, caffeine and gas-producing oligosaccharide containing-foods like beans, cabbage, and onions) is equally as effective as a low-FODMAP diet.[277] Further randomised comparative dietary trials are now needed aiming to address not only the impact of diet on IBS symptoms but also assessing extra-intestinal symptoms, social-QOL, day-to-day practicality, sustainability, and safety. Indeed, restriction of the oligosaccharide (fructan) content has been shown to alter the composition of beneficial colonic microbiota.[278-281] With regards to the low-FODMAP diet reductions in the proportions of *Bifidobacteria*, butyrate-producing *Clostidrial* groups and mucus-associated bacterium *Akkermansia muciniphila* have been noted in IBS patients and healthy controls.[278,279] A GFD has been shown to reduce *Bifidobacteria* and *Lactobacilli* in healthy subjects.[280,281] The implications of altered microbiome on long-term colonic health are unknown and require elucidation.

Nevertheless, dietary therapies are now on the menu for treating D-IBS.[267] Some investigators have proposed that it may be reasonable to trial a GFD before a low-FODMAP diet.[160] This suggestion would be supported by the fact that a GFD is readily-available, popular, and has seen a dramatic rise in awareness amongst the public and chefs over the last decade.[170,282] Moreover, dietitians are experienced and generally accustomed to providing a GFD which is not yet the case for a low-FODMAP diet in view of its recent

introduction to medical practice and that it requires additional specialist cost-incurred training for optimal delivery and efficacy.[283] Finally, a GFD also carries a partial low-FODMAP effect (as wheat contains fructans) making it seem plausible to start with this diet first before progressing to the wider repertoire of excluding all-FODMAP components should symptoms persist. Indeed, it has been shown that individuals with self-reported NCGS, when still symptomatic on a GFD, reap further benefit when placed on a low-FODMAP diet.[158]

In line with this, we speculate that the pathophysiological mechanism by which a GFD improves the symptoms of D-IBS may differ according to HLA-DQ status. It has previously been shown that D-IBS subjects have increased small bowel intestinal permeability compared to controls,[284] and that duodenal instillation of dietary food antigens (commonly to wheat) leads to an immediate and transient increase in duodenal intraepithelial lymphocyte density, formation of epithelial leaks/gaps, and widening of intervillous spaces as seen by confocal laser endosmicroscopy.[285] Elsewhere, it has been shown that HLA-DQ2/8 positive D-IBS subjects have faster small bowel transit, with exposure to a gluten-containing diet reducing tight-junction proteins and increasing intestinal permeability, compared to HLA-DQ2/8 negative subjects.[175,268] Further, experimental models in HLA-DQ8 gluten sensitised mice have provided a mechanistic explanation for symptom induction by demonstrating gliadin to induce immune activation in the absence of intestinal atrophy, paralleled with increased acetylcholine release from the myenteric plexus resulting in enhanced muscle contractility and epithelial hyper-secretion, with the abnormalities reversed following gluten withdrawal.[178] To translate this into clinical practice, it may therefore be that in our study the effect of a GFD in HLA-DQ2/8 positive subjects can be attributed to the specific removal of gluten-protein per se, thereby “switching-off” a HLA-

DQ2/8 driven immune-mediated process and restoring gut health. This could account for the improvement seen in D-IBS symptoms but also explain the marked benefit experienced from a generalised and mental well-being perspective in the HLA-DQ2/8 positive group; in that, gluten-related or equivalent protein-peptides (exorphins) are no longer present or able to cross the intestinal epithelium into the systemic circulation and central nervous system where they may cause symptoms such as depression.[286] In contrast, for the HLA-DQ2/8 negative D-IBS group the improvement seen with a GFD may not be due to removal of the gluten-protein per se but rather the fructan component. This suggestion would be supported by rapid resolution of intestinal distension seen in this group suggesting the fermentable carbohydrates may have been responsible due to their effects on gaseous production.[57] Further research studies, using small bowel permeability testing and magnetic resonance intestinal imaging, should now aim to assess the effects of specifically isolated gluten-based based constituents in D-IBS subjects according to HLA-DQ2/8 status. However, in clinical practice our findings do not recommend the routine use of HLA-DQ typing in D-IBS patients as symptomatic improvements to a GFD were seen amongst both groups.

6.6 Conclusion

A dietitian led GFD should be considered as a therapeutic option for the management of D-IBS patients who are previously naïve to the effects of gluten. A clinical response was seen in 71% of patients undertaking a six-week GFD, with 72% of these opting to continue with a GFD for the foreseeable future. At 18-month mean follow-up such patients demonstrated that the diet is durable, maintains symptom remission, and does not carry detrimental effect towards body-mass index or biochemical status. The pathophysiological mechanism may differ according to HLA-DQ2/8 status and warrants further study.

CHAPTER 7: Summary of key findings and recommendations for future research

Interest in developing and pursuing this thesis was based upon media reports repeatedly suggesting a huge surge in public demand for gluten-free products despite individuals apparently not having a diagnosis of either coeliac disease or IgE-wheat allergy.

We have established that there has been a dramatic change in societal awareness of gluten-related disorders over the last decade. Historically, knowledge of coeliac disease and gluten sensitivity was limited amongst the general public and particularly chefs in the year 2003. However, by the year 2013 there has been a marked increase with similar awareness amongst both groups. This knowledge may have been acquired through increasing media advertisements regarding the gluten-free lifestyle, consumer/household demands for a gluten-free diet, and changes in the European food legislation now requiring businesses to clearly provide and display allergy information (which includes gluten) on unpackaged foods. It can be envisaged that knowledge and awareness of gluten-related disorders and the gluten-free diet will continue to grow.

This suggestion would be supported by our subsequent study showing that a significant proportion of the general public are self-reporting gluten sensitivity and taking a gluten-free diet of their own volition even in the absence of a doctor-diagnosis of either coeliac disease or IgE-wheat allergy. The characteristic phenotype of such individuals is young to middle-aged women describing a constellation of intestinal and extra-intestinal symptoms following ingestion of gluten-based products. The intestinal symptoms are consistent with that of irritable bowel syndrome and the extra-intestinal symptoms commonly include fatigue,

headaches, depression, skin rash and joint pains. When investigated in secondary-care gastroenterology practice we identified that the majority did not reach a diagnosis of coeliac disease or IgE-wheat allergy. Instead, these individuals could be termed as non-coeliac gluten sensitivity following recent double-blind placebo-controlled studies. We also demonstrated that subjects with non-coeliac gluten sensitivity are less likely to suffer nutritional complications or low body-mass index compared to coeliac disease. This can be explained by the relatively normal villous architecture in non-coeliac gluten sensitivity allowing adequate small bowel surface area for absorption, as opposed to the villous atrophy seen in coeliac disease. Nevertheless, it has been shown elsewhere that subjects with non-coeliac gluten sensitivity have lower bone mineral density compared to healthy controls and it would therefore be useful to assess for osteoporosis over longitudinal follow-up. It also remains to be established whether non-coeliac gluten sensitivity is a transient or permanent phenomenon. Indeed, its cause is unknown and the role of gastrointestinal infections, microbiota profile, and intestinal permeability will be of future interest.

Furthermore, it should be taken into consideration that when interpreting studies on gluten sensitivity there are areas fraught with complexity and uncertainty. Firstly, adequately differentiating between coeliac disease and non-coeliac gluten sensitivity is not straightforward. A negative HLA-DQ2/8 status can exclude CD due to its 100% negative predictive value. However, if positive or not available then a gluten-challenge is required for which there is no international consensus on optimal dosage and duration.[217] A potentially more favourable/tolerable option which could help address this ambiguity is the use of *in-vitro* gliadin challenge but this is yet to be routinely adopted. In addition, whether we should be diagnosing gluten-sensitive patients with isolated raised duodenal intraepithelial

lymphocytes yet negative coeliac serology as belonging to the spectrum of non-coeliac gluten sensitivity is controversial.[217] It has recently shown that a proportion of such patients will have endomysial and/or anti-TTG-2 antibodies on the duodenal culture medium, thereby rather supporting a diagnosis of coeliac disease over non-coeliac gluten sensitivty.[154,287-290] Unfortunately, testing for duodenal culture antibodies is not routinely available. Hence, we believe such patients should be viewed with an open-mind especially when critiquing the numerous studies evaluating non-coeliac gluten sensitivity and maybe we should consider re-challenging these patients with gluten a few years later to see if they go on to develop any signs of overt coeliac disease. In the interim period it would be useful to determine whether these so-called non-coeliac gluten sensitivity patients with raised duodenal intraepithelial lymphocytes clinically differ from those with normal biopsies.

Secondly, despite double-blind placebo-controlled studies being considered as the gold-standard method in subsequently confirming non-coeliac gluten sensitivity they are cumbersome to perform and not readily available in clinical practice. In addition, they must be viewed with some caution given that associated constituents found in wheat such as fructans (belonging to the fermentable carbohydrate [FODMAP] family), amylase-trypsin inhibitors, and wheat-germ agglutinins may be responsible symptom triggers instead. Unfortunately, no diagnostic markers currently exist to differentiate between placebo, gluten, and non-gluten components. Some investigators have proposed that antigliadin antibodies potentially support the gluten theory although they are limited by low sensitivity and specificity. Another promising tool could be the use of confocal laser endomicrosopy which can demonstrate real-time in-vivo immune-mediated reactions in the duodenum upon instilling gluten-based products in patients with irritable bowel syndrome. This

innovative technique is not yet routinely available but might prove to be a major breakthrough in at least excluding fructans whose effects can be attributed to fermentation as opposed to immune-mediated. However, it will not be able to differentiate gluten from non-gluten proteins and furthermore uncertainty will still arise in the HLA-DQ2 and/or HLA-DQ8 positive cohort as such changes will be expected to also occur in coeliac disease. It is therefore not surprising that at a meeting held in 2014 among experts in the field of gluten-related disorders the panel regularly raised the issue that future studies and identification of biomarkers in non-coeliac gluten sensitivity need to be performed in individuals who are HLA-DQ2 and HLA-DQ8 negative so that potential ambiguity with coeliac disease can be avoided.[291] Hence, in the current climate some of these experts propose that we should be using the terms "self-reported" non-coeliac gluten sensitivity, or "non-coeliac wheat sensitivity", or even "patients who avoid wheat and/or gluten".

Moving on, we established that the majority of patients with self-reported non-coeliac gluten sensitivity appear to belong to the spectrum of dietary-related irritable bowel syndrome. However, a small minority had an underlying diagnosis of inflammatory bowel disease; such patients provided clues in their clinical history and/or laboratory markers which prompted the necessary colonic investigations. This novel finding was of interest particularly as around the same time studies elsewhere suggested there to be a high use of a gluten-free diet in patients with inflammatory bowel disease. We sought to explore this further and confirmed these findings but also established that inflammatory bowel disease patients self-reporting gluten sensitivity were more likely to have severe/stricturing disease compared to those without gluten-sensitivity. The pathophysiological mechanism remains to be elucidated but may be a cause or consequence effect of gluten-based products. Future

studies in this area need to determine HLA-DQ2/8 status, presence of *Saccharomyces Cerevisiae* antibodies, and also randomise inflammatory bowel disease patients to gluten and FODMAPs similar to that already being performed for irritable bowel syndrome.

Finally, given that diarrhoea-predominant irritable bowel syndrome is extremely prevalent in the out-patient gastroenterology setting we sought to determine whether a gluten-free diet could be a viable treatment option for patients previously naïve to the effects of gluten. We established that a gluten-free diet provides a sustained reduction in intestinal and extra-intestinal symptoms of diarrhoea-predominant irritable bowel syndrome without any detriment to anthropometric status or body-mass index. Furthermore, variations in the type of clinical response were noted according to HLA-DQ status and future studies would benefit from establishing the effects of gluten and FODMAPs with regards to intestinal permeability and gas production depending on HLA-genotype. Nevertheless, our findings have added to the recent hotbed of literature supporting that diet is on the menu to treat irritable bowel syndrome. The effectiveness and social acceptability of a gluten-free diet needs to be compared against a low-FODMAP diet. Furthermore, as these diets can be associated with alterations in colonic microbiota long-term assessment of colonic health is needed.

In summary, self-reported gluten sensitivity and the use of a gluten-free diet exists outside of coeliac disease and IgE-wheat allergy. There is a common association with irritable bowel syndrome although it can be seen in inflammatory bowel disease. This clinical entity has been termed non-coeliac gluten sensitivity yet must be viewed with caution given its current lack of diagnostic biomarkers and the complex heterogeneous nature of gluten-based products. Hence, patients presenting with self-reported non-coeliac gluten sensitivity must be counselled about the uncertainties surrounding their diagnosis.

APPENDICES OF QUESTIONNAIRRES

Appendix A: Public questionnaire on awareness of gluten related disorders

Sex Age Date of Birth

Have you heard of Peanut allergy? Yes No Don't know

Have you heard of Coeliac disease? Yes No Don't know

Have you heard of Gluten sensitivity? Yes No Don't know

How many people do you think are affected by peanut allergy?

1 in 10 1 in 50 1 in 100 1 in 200 1 in 500 1 in 1000 1 in 5000

How many people do you think are affected by coeliac disease?

1 in 10 1 in 50 1 in 100 1 in 200 1 in 500 1 in 1000 1 in 5000

How many people do you think are affected by gluten sensitivity?

1 in 10 1 in 50 1 in 100 1 in 200 1 in 500 1 in 1000 1 in 5000

Do you recognise this symbol? Yes No

If yes, what does It stand for…….

Appendix B : Chef questionnaire on awareness of gluten related disorders

Sex **Age** **Date of Birth**

Have you heard of Peanut Allergy? Yes No Don't know

Have you heard of Coeliac Disease? Yes No Don't know

Have you heard of Gluten sensitivity? Yes No Don't know

How many people do you think are affected by peanut allergy?

1 in 10 1 in 50 1 in 100 1 in 200 1 in 500 1 in 1000 1 in 5000

How many people do you think are affected by coeliac disease?

1 in 10 1 in 50 1 in 100 1 in 200 1 in 500 1 in 1000 1 in 5000

How many people do you think are affected by gluten sensitivity?

1 in 10 1 in 50 1 in 100 1 in 200 1 in 500 1 in 1000 1 in 5000

Where do they work Restaurant Take away

Are there any Gluten Free Signs on the menu or outside? Yes No

Do you recognise this symbol? Yes No

If yes, what does It stand for……

Were you trained (degree, diploma, course etc) – when did you graduate……..

Yes No

How many years of service have you given to the food industry …….

Have you heard of Coeliac UK's accreditation scheme for business Yes No

Average cost of meal (per person)

£0-5 £5-10 £10-20 £20-40 >£40

Appendix C: Questionnaire on self-reported gluten sensitivity

Dear Sir/Madam

We would be grateful if you would kindly complete this 1-5 minute questionnaire survey as part of a research project being undertaken by the Gastroenterology department at the Royal Hallamshire Hospital, Sheffield. There are two parts to this questionnaire and although it asks about your bowel symptoms and past medical history, it is anonymous and the results are confidential and will be used only for research purposes. This questionnaire has been registered with the Sheffield Teaching Hospitals. You may find parts of this questionnaire repetitive but please try and answer all the appropriate questions tailored for you. Should you have any queries or difficulties completing this survey, please ask our helpful young student doctors!

Thank you for your co-operation and time in completing this questionnaire

Part 1: This asks for basic information about yourself, any abdominal symptoms and your general state of health

Q1) Age _____ D.O.B_____

Q2) Male ☐ or Female ☐

Q3) Employed ☐, Unemployed ☐, Disabled ☐, Retired ☐

Q4) Single ☐, In a relationship ☐, Divorced ☐, Widowed ☐

Q5) Race : White ☐, Black ☐, Asian ☐, Other ☐ (please state_____)

Q6) Post code _____

Q7) Have you suffered with episodes of abdominal pains or discomfort for the last 6 months or more? Yes ☐ No ☐ - **- if No, please go to Q16**

Q8) If yes, <u>how many days in a month</u> do you approximately experience these abdominal pains or discomfort?

One day a month ☐ Four days a month ☐

Two days a month ☐ 5-10 days a month ☐

Three days a month ☐ More than 10 days a month ☐

Q9) Do you suffer from abdominal bloating (feeling full of gas)? Yes ☐ No ☐

Q10) Do you feel an improvement in your abdominal pains or discomfort after you have emptied your bowels? Yes ☐ No ☐

Q11) Was the start of your abdominal pains or discomfort associated with a change in your bowel frequency? Yes ☐ No ☐

Q12) If yes to Q11, how would you best describe the predominant change in bowel habit?

Diarrhoea ☐ , Constipation ☐ , Alternates between Diarrhoea & Constipation ☐

Q13) Was the start of your abdominal pains or discomfort associated with a change in stool consistency? Yes ☐ No ☐

Q14) If yes to Q13, how best would you describe your stool motions?

Loose, watery or sloppy ☐ Hard, pellet like ☐
Combination of loose at times and hard other times ☐

Q15) Do your abdominal and bowel symptoms get worse with stress? Yes ☐ No ☐

Q16) Are you known to suffer from any of the following? (tick as many that apply)

Anxiety	☐	Chronic headaches	☐
Depression	☐	Nut allergy	☐
Bipolar disorder	☐	Egg allergy	☐
Schizophrenia	☐	Dairy product intolerance	☐
Thyroid disease	☐	Bowel cancer	☐
Young onset diabetes (childhood/early adulthood)	☐	Stomach cancer	☐
Pernicious anaemia (low vitamin B12)	☐	Irritable bowel syndrome	☐
Chronic fatigue	☐	Coeliac disease	☐
Fibromyalgia	☐	Inflammatory bowel disease	☐
ME	☐	Heartburn/reflux	☐

Part 2 – Gluten related symptoms

This part of the questionnaire focuses on whether you develop problems when you eat gluten. Gluten is a product found in wheat, barley or rye. Therefore, it is found in common everyday diets such as cereal, bread, cakes, biscuits, pasta, pizza etc. Should you have any queries or difficulties completing this survey, please ask our helpful young student doctors!

Q1) Do you experience any of the following symptoms which you relate to eating gluten based products? (tick as many that apply)

Bloating (feel full of air)	☐	Headaches	☐
Abdominal Pain	☐	Mental confusion	☐
Abdominal discomfort	☐	Lack of co-ordination	☐
Diarrhoea	☐	Numbness/pins & needles	☐
Constipation	☐	Lack of energy	☐
Belching	☐	Skin Rash	☐
Flatulence	☐	Joint Pains	☐
Sickness	☐	Anaemia	☐

Others (please specify) _____

If you do not suffer from any gluten related symptoms, do not proceed. Thank you

Q2) If yes, how often do you experience symptoms after eating gluten products?

Every time I eat gluten products	☐	Few times a month	☐
On most occasions/days	☐	Few times a year	☐
Few days a week	☐		

Q3) How soon after eating gluten products do you develop symptoms?

Almost immediately (less than one hour)	☐	The next day	☐
1-6 hours later	☐	A few days later	☐
6-24 hrs later	☐		

Q4) How long do your symptoms generally last for?

Minutes ☐ Hours ☐ Days ☐ Weeks ☐ Months ☐

Q5) Which gluten product(s) seems to cause problems? (tick as many that apply)

Bread ☐	Pizza ☐	Cakes ☐
Cereal ☐	Pasta ☐	Biscuits ☐
Porridge ☐	Others (please state) _____	

Q6) How long have you had a problem related to gluten?

(state approximate number) _____ months or _____years

Q7) Have you ever seen a healthcare professional due to problems related to gluten? Yes ☐ No ☐

Q8) If yes, please state whom you have seen? (tick as many that apply)

GP ☐ , Hospital doctor ☐, dietician ☐, other ☐(please state) _____

Q9) If yes, have you undergone any of the following tests to look <u>specifically</u> for a cause as to why you have problems related to gluten? (tick as many that apply)

Coeliac blood test	Yes ☐	No ☐	Not sure ☐
Skin prick allergy test	Yes ☐	No ☐	Not sure ☐
Endoscopy (camera into stomach)	Yes ☐	No ☐	Not sure ☐
You have had no tests at all	Yes ☐		

Other tests (please state) _____

Q10) If yes to Q7, Q8, Q9 have you been given any of the following diagnosis? (please ask the student doctor to explain the different conditions in more detail)

Coeliac disease☐, Wheat allergy ☐, Coeliac disease has been excluded ☐,

No explanation given ☐ , You are not sure ☐, other ☐ _____

Q11) Have your ever tried a gluten free diet?	Yes ☐	No ☐
Q12) If yes, was it beneficial for your symptoms?	Yes ☐ No ☐	Not sure ☐
Q13) If yes to Q11, Are you still on a gluten free diet ?	Yes ☐	No ☐

Thank You – there are no further questions

Appendix D: Crohn's Disease Activity Index (CDAI) Score[235]

1. No of liquid or soft stools past 7 days (total value X 2=_____)

Day 1	D2	D3	D4	D5	D6	D7

2. Abdominal pain last 7 days [none 0, mild 1, moderate 2, severe 3] (total value X 5 = ____)

D1	D2	D3	D4	D5	D6	D7

3. **General well being last 7 days [well 0, slightly unwell 1, poor 2, very poor 3, terrible 4]**

(total value X 7= _____)

D1	D2	D3	D4	D5	D6	D7

4. Any extra-intestinal symptoms [1 point for each] total value X 20=_____

Arthritis/athralgia ☐ , iritis/uveitis ☐ , skin/mouth lesions ☐ ,

perianal disease (fissure, fistula, abscess) ☐ , other fistula ☐ , T > 37.8 in last wk ☐

5. Are you taking loperamide or opiates for diarrhoea? ☐ **(total value X 30=_____)**

6. Abdominal mass? (total value X 10=_____)

None (0 points) ☐ , Questionable (2 points) ☐ , Definite (5 points) ☐

7. Enter current haematocrit (HCT): for male (47- HCT), for female (42- HCT)

[total valuex6=_____]

8. Weight (current in kg ____) and (standard in kg_____)

Do the following calculation 100 x (1-current/standard)= _____

9. Work out CDAI score =

Appendix E: Simple Colitis Activity Index (SCAI) Score[236,237]

Q1. Stools per day	1-3 ☐ (0 points),		7-9 ☐ (2 points)	
	4-6 ☐ (1 point)		> 9 ☐ (3 points)	
Q2. Stools per night	None ☐ (0 points)	1-3 ☐ (1 point)	4-6 ☐ (2 points)	
Q3. Urgency	None ☐ (0 points)		Immediate ☐ (2 points)	
	Hurry ☐ (1 point)		Incontinence ☐ (3 points)	
Q4. PR blood	None ☐ (0 points)			
	Trace ☐ (1 point)			
	Occasionally frank ☐ (2 points)			
	Usually frank ☐ (3 points)			
Q5. General well being	Very well ☐ (0 points)			
	Slightly below par ☐ (1 point)			
	Poor ☐ (2 points)			
	Very poor ☐ (3 points)			
	Terrible ☐ (4 points)			

Q6) Extraintestinal symptoms (1 point for each)

Arthritis	☐
Pyoderma gangrenosum	☐
Erythema Nodosum	☐
Uveitis	☐

TOTAL SCAI SCORE = _____

Appendix F: Irritable Bowel Syndrome Symptom Severity Score (IBS-SSS) [269]

Patient details

Week ….

Instructions

The form is designed to enable us to record and monitor the severity of your irritable bowel syndrome (IBS). It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (i.e. over the last 10 days or so). All information will be kept in **strict** confidence.

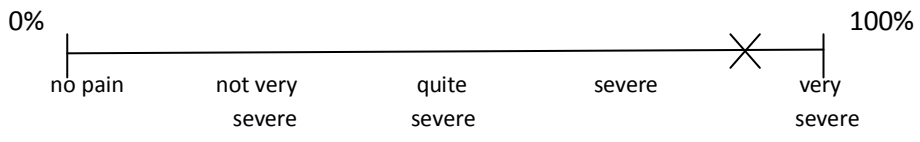
1. For questions where a number of different responses are a possibility please circle the response appropriate to you.
2. Some questions will require you to write in an appropriate response
3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem

For example:

How severe was your pain?

Please place our cross (X) anywhere on the line between 0-100% in order to indicate as accurately as possible the severity of your symptoms

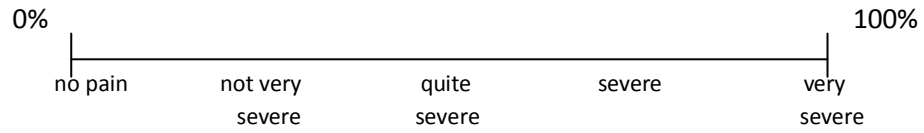
This example shows a severity of approximately 90%



1. a) Do you currently suffer from abdominal (tummy) pain? YES NO

Circle appropriate box

b) If yes, how severe is your abdominal (tummy) pain?



c) Please enter the number of days that you get pain in every 10 days. *For example if you enter 4 it means you get pain 4 out of 10 days. If you get pain everyday enter 10.*

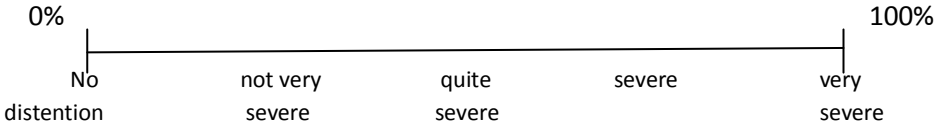
Number of days with pain ☐ x10

2 a) Do you currently suffer from abdominal distention?* YES NO

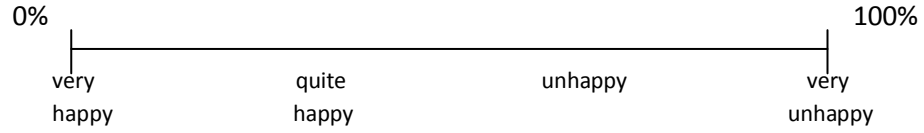
(bloating, swollen or tight tummy) *Circle appropriate box*

*(*women, please ignore distention related to your periods)*

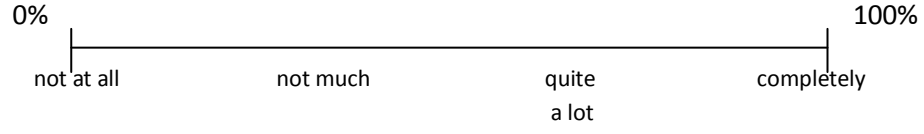
b) If yes, how severe is your abdominal distention/tightness?



3. How satisfied are you with your bowel habit?



4. Please indicate with a cross on the line below how much your irritable bowel syndrome is affecting or interfering with your life in general



IBS Severity Score ☐

Appendix G: Hospital Anxiety and Depression Scale (HADS)[270]

This questionnaire helps your physician to know how you are feeling. Read every sentence. Place an "X" on the answer that best describes how you have been feeling during the LAST WEEK. You do not have to think too much to answer. In this questionnaire, spontaneous answers are more important

A	**I feel tense or 'wound up':**	
	Most of the time	3
	A lot of the time	2
	From time to time (occ.)	1
	Not at all	0
D	**I still enjoy the things I used to enjoy:**	
	Definitely as much	0
	Not quite as much	1
	Only a little	2
	Hardly at all	3
A	**I get a sort of frightened feeling as if something awful is about to happen:**	
	Very definitely and quite badly	3
	Yes, but not too badly	2
	A little, but it doesn't worry me	1
	Not at all	0
D	**I can laugh and see the funny side of things:**	
	As much as I always could	0
	Not quite so much now	1
	Definitely not so much now	2
	Not at all	3
A	**Worrying thoughts go through my mind:**	
	A great deal of the time	3
	A lot of the time	2
	From time to time, but not often	1
	Only occasionally	0
D	**I feel cheerful:**	
	Not at all	3
	Not often	2
	Sometimes	1
	Most of the time	0
A	**I can sit at ease and feel relaxed:**	
	Definitely	0
	Usually	1
	Not often	2
	Not at all	3

D	**I feel as if I am slowed down:**	
	Nearly all the time	3
	Very often	2
	Sometimes	1
	Not at all	0
A	**I get a sort of frightened feeling like "butterflies" in the stomach:**	
	Not at all	0
	Occasionally	1
	Quite often	2
	Very often	3
D	**I have lost interest in my appearance:**	
	Definitely	3
	I don't take as much care as I should	2
	I may not take quite as much care	1
	I take just as much care	0
A	**I feel restless as I have to be on the move:**	
	Very much indeed	3
	Quite a lot	2
	Not very much	1
	Not at all	0
D	**I look forward with enjoyment to things:**	
	As much as I ever did	0
	Rather less than I used to	1
	Definitely less than I used to	2
	Hardly at all	3
A	**I get sudden feelings of panic:**	
	Very often indeed	3
	Quite often	2
	Not very often	1
	Not at all	0
D	**I can enjoy a good book or radio/TV program:**	
	Often	0
	Sometimes	1
	Not often	2
	Very seldom	3

Appendix H: Fatigue Impact Scale (FIS)[271]

Below is a list of statements that describe how fatigue may cause problems in people's lives. Please read each statement carefully. Circle one number that best indicates how much of a problem fatigue has been for you these **past four (4) weeks**, including today. Please mark one box for each statement and do not skip any statements.

Circle one number on each line	No Problem	Small Problem	Moderate Problem	Big Problem	Extreme Problem
1. *Because of my fatigue,* **I feel less alert**.	0	1	2	3	4
2. *Because of my fatigue,* **I feel that I am more isolated from social contact.**	0	1	2	3	4
3. *Because of my fatigue,* **I have had to reduce my workload or responsibilities.**	0	1	2	3	4
4. *Because of my fatigue,* **I am more moody**.	0	1	2	3	4
5. *Because of my fatigue,* **I have difficulty in paying attention for a long period of time.**	0	1	2	3	4
6. *Because of my fatigue,* **I feel as if I cannot think clearly.**	0	1	2	3	4
7. *Because of my fatigue,* **I work less effectively (this applies to work inside or outside the home).**	0	1	2	3	4
8. *Because of my fatigue,* **I have to rely more on others to help me or do things for me.**	0	1	2	3	4
9. *Because of my fatigue,* **I have difficulty in planning activities in advance because my fatigue may interfere with them.**	0	1	2	3	4
10. *Because of my fatigue,* **I am more clumsy and uncoordinated.**	0	1	2	3	4
11. *Because of my fatigue,* **I find that I am more forgetful.**	0	1	2	3	4
12. *Because of my fatigue,* **I am more irritable and get angry more easily.**	0	1	2	3	4
13. *Because of my fatigue,* **I have to be careful about pacing my physical activities.**	0	1	2	3	4
14. *Because of my fatigue,* **I am less motivated to do anything that requires physical effort.**	0	1	2	3	4
15. *Because of my fatigue,* **I am less motivated to engage in social activities.**	0	1	2	3	4
16. *Because of my fatigue,* **my ability to travel outside my home is limited.**	0	1	2	3	4
17. *Because of my fatigue,* **I have trouble maintaining physical effort for long periods**	0	1	2	3	4
18. *Because of my fatigue,* **I find it difficult to make decisions.**	0	1	2	3	4
19. *Because of my fatigue,* **I have few social contacts outside my own home.**	0	1	2	3	4

20. *Because of my fatigue,* **normal day-to-day events are stressful for me.**	0	1	2	3	4

21. *Because of my fatigue,* **I am less motivated to do anything that requires thinking.**	0	1	2	3	4
22. *Because of my fatigue,* I **avoid situations that are stressful for me.**	0	1	2	3	4
23. *Because of my fatigue,* **my muscles feel much weaker than they should.**	0	1	2	3	4
24. *Because of my fatigue,* **my physical discomfort is increased.**	0	1	2	3	4
25. *Because of my fatigue,* **I have difficulty dealing with anything new.**	0	1	2	3	4
26. *Because of my fatigue,* **I am less able to finish tasks that require thinking.**	0	1	2	3	4
27. *Because of my fatigue,* **I feel unable to meet the demands that people place on me.**	0	1	2	3	4
28. *Because of my fatigue,* **I feel less able to provide financial support for myself and my family.**	0	1	2	3	4
29. *Because of my fatigue,* **I engage in less sexual activity.**	0	1	2	3	4
30. *Because of my fatigue,* **I find it difficult to organise my thoughts when I am doing things at home or at work.**	0	1	2	3	4
31. *Because of my fatigue,* **I am less able to complete tasks that require physical effort.**	0	1	2	3	4
32. *Because of my fatigue,* **I worry about how I look to other people.**	0	1	2	3	4
33. *Because of my fatigue,* **I am less able to deal with emotional issues.**	0	1	2	3	4
34. *Because of my fatigue,* **I feel slowed down in my thinking.**	0	1	2	3	4
35. *Because of my fatigue,* **I find it hard to concentrate.**	0	1	2	3	4
36. *Because of my fatigue,* **I have difficulty in participating fully in family activities.**	0	1	2	3	4
37. *Because of my fatigue,* **I have to limit my physical activities.**	0	1	2	3	4
38. *Because of my fatigue,* **I require more frequent or longer periods of rest.**	0	1	2	3	4
39. *Because of my fatigue,* **I am not able to provide as much emotional support to my family as I should.**	0	1	2	3	4
40. *Because of my fatigue,* **minor difficulties seem like major difficulties.**	0	1	2	3	4

Appendix I: Short Form 36 Quality of Life Health Survey (SF-36)[272]

SF-36 Health Survey

INSTRUCTIONS: This survey asks your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Please answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:

(circle one)

Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. Compared to one year ago, how would you rate your health in general now?

(circle one)

Much better now than one year ago	1
Somewhat better than one year ago	2
About the same as one year ago	3
Somewhat worse than one year ago	4
Much worse now than one year ago	5

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(circle one number on each line)

Activities	Yes, limited a lot	Yes, limited a little	No, not limited at all
Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.	1	2	3
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
Lifting or carrying groceries	1	2	3
Climbing several flights of stairs	1	2	3
Climbing one flight of stairs	1	2	3
Bending, kneeling or stooping	1	2	3
Walking more than a mile	1	2	3
Walking half a mile	1	2	3
Walking one hundred yards	1	2	3
Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

(circle one number on each line)

	Yes	No
Cut down on the amount of time you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Were limited in the kind of work or other activities	1	2
Had difficulty performing the work or other activities (for example, it took extra effort	1	2

5.During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

(circle one number on each line)

	Yes	No
Cut down on the amount of time you spent on work or other activities	1	2
Accomplished less than you would like	1	2
Didn't do work or other activities as carefully as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours or groups?

(circle one)

Not at all	1
Slightly	2
Moderately	3
Quite a bit	4
Extremely	5

7. How much bodily pain have you had during the past 4 weeks?

(circle one)

None	1
Very mild	2
Mild	3
Moderate	4
Severe	5
Very severe	6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(circle one)

Not at all	1
A little bit	2
Moderately	3
Quite a bit	4
Extremely	5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
Did you feel full of life?	1	2	3	4	5	6
Have you been a very nervous person?	1	2	3	4	5	6
Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
Have you felt calm and peaceful?	1	2	3	4	5	6
Did you have a lot of energy?	1	2	3	4	5	6
Have you felt downhearted and low?	1	2	3	4	5	6
Did you feel worn out?	1	2	3	4	5	6
Have you been a happy person?	1	2	3	4	5	6
Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

(circle one)

All of the time 1
Most of the time 2
Some of the time 3
A little of the time 4
None of the time 5

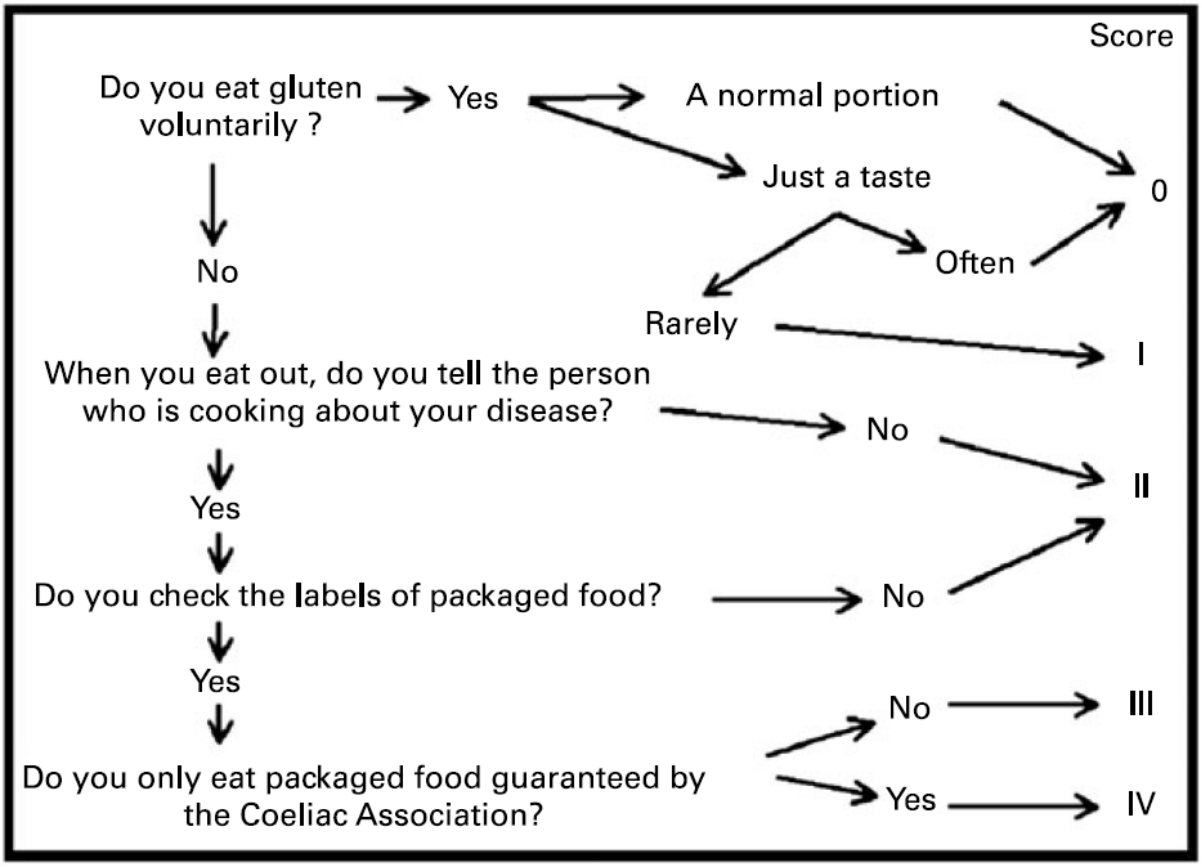
11. How TRUE or FALSE is each of the following statements to you?

(circle one number on each line)

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
I seem to get ill more easily than other people	1	2	3	4	5
I am as healthy as anybody I know	1	2	3	4	5
I expect my health to get worse	1	2	3	4	5
My health is excellent	1	2	3	4	5

Appendix J: Gluten Free Dietary Adherence[274]

To be completed by dietitian



Validated questionnaire and scoring system to assess compliance with a gluten-free diet.

"Often": the patient consumes gluten so often that he/she cannot remember when and how many times that happened.

"Rarely": the patient consumes gluten occasionally. She/he can remember when and how many times that has happened

APPENDICES - GENERAL

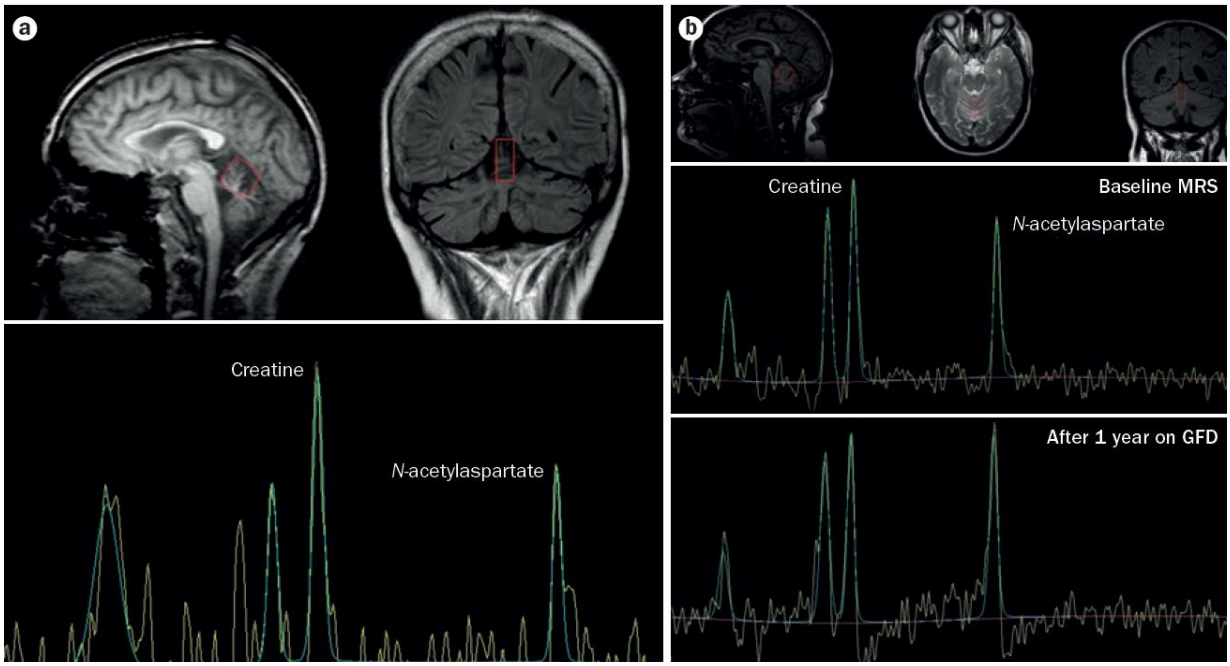
Appendix A1: Cross-sectional population-based observational studies assessing the use of a gluten-free diet and known diagnosis of coeliac disease [170]

Author	Year of publication	Country	Group	Sample size	Avoidance of gluten-based products	Known previous diagnosis of CD
Tanpowpong *et al*[194]	2012	New Zealand	Children – general population	916	5% (n=48)	1% (n=9)
Rubio-Tapia *et al*[195]	2013	US	Age ≥6years, NHANES 2009-10	7,798	0.63% (n=55)	0.1% (n=6)
Aziz *et al*[196]	2014	UK	Adults – general population	1,002	3.7% (n=37)	0.8% (n=8)
Lis *et al*[197]	2014	Australia	Adults- athletes	910	41.2% (n=375)	None
Golley *et al*[198]	2015	Australia	Adults-general population	1,184	10.6% (n=126)	1.2% (n=14)
Mardini *et al*[199]	2015	US	Age ≥6 years, NHANES 2009-10 & 2011-12 data combined	14,701	0.9% (n=142)	0.1% (n=21)
Ontiveros et al[200]	2015	Mexico	Adults-general population	1,238	3.7% (n=45)	0.08 (n=1)
Abbreviations; GFD, gluten-free diet; NHANES, National Health and Nutrition Examination Survey						

Appendix B1: Characteristic symptoms with self-reported NCGS reported from multiple centres [126,179,180,196]

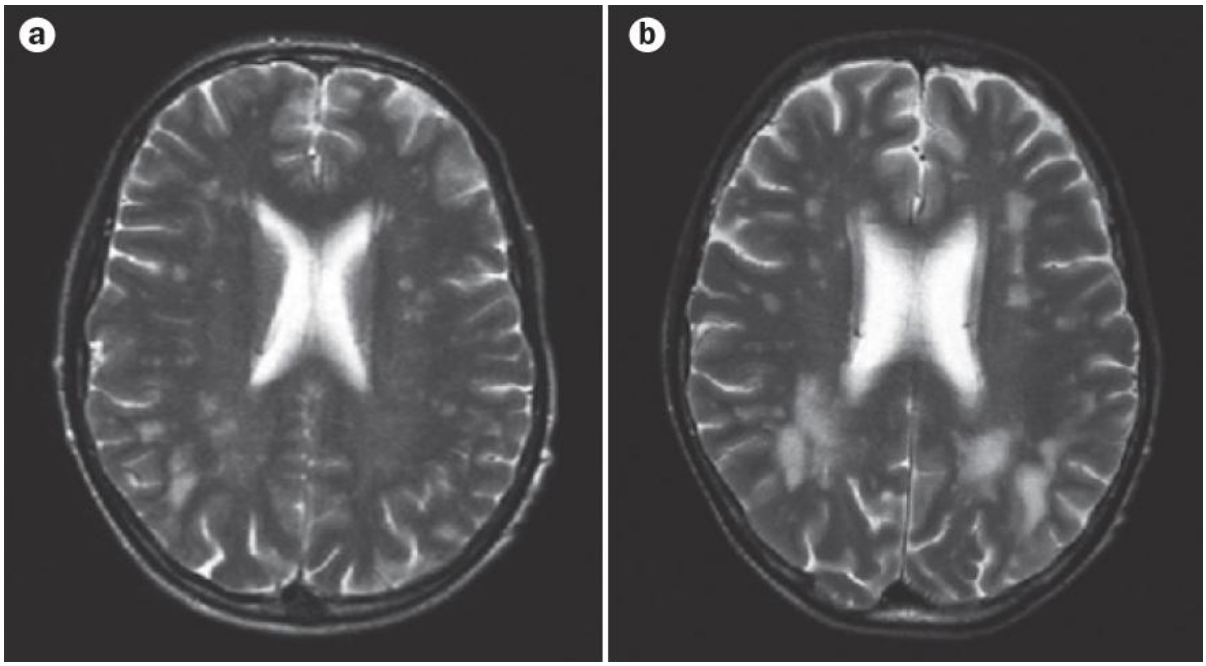
Lower Gastrointestinal symptoms	Upper Gastrointestinal symptoms	Extra-intestinal symptoms
Diarrhoea: 16-54% Constipation: 18-24% Altered bowel habit: 27% Abdominal pain/discomfort: 67-83% Bloating: 72-87% Weight loss: 25%	Epigastric pain: 52% Nausea: 9-44% Aerophagia: 36% Gastro-oesophageal reflux: 32% Apthous stomatatis: 31%	Skin rash (eczema or dermatitis): 6-40% Depression: 15-22% Confusion: 5% Foggy mind: 34-42% Anxiety: 39% Headaches: 22-54% Limb numbness: 6-32% Joint/muscle pains: 8-31% Fatigue: 23-64% Lack of well-being: 68%

Appendix C1: Images of gluten ataxia



MRS of the cerebellum in patients with ataxia secondary to NCGS. These patients had positive AGA but no evidence of enteropathy. The voxel was placed in the cerebellar vermis, which is primarily affected in gluten ataxia. In healthy individual the ratio of N-acetylaspartate:creatine should be >1. a| In the first patient the ratio of N-acetylaspartate:creatine was 0.56, which was markedly reduced. b| In the second patient the N-acetylaspartate:creatine improved from 0.65 to 1.01 after 1 year on a GFD. This increase was associated with clinical improvement of the ataxia. Abbreviations: AGA, antigliadin antibodies; GFD, gluten-free diet; MRS, magnetic resonance spectroscopy. (*With permission and courtesy of Professor Marios Hadjivassiliou, Royal Hallamshire Hospital, Sheffield).*[170]

Appendix D1: Images of gluten encephalopathy



A head MRI of a patient with gluten encephalopathy. A 55-year-old patient with intractable headaches and positive AGA (gluten encephalopathy), but no evidence of enteropathy. Initial adherence to a GFD was associated with improvement of the headaches but the patient was unable to adhere to the diet after 3 months. The left scan at baseline shows white matter abnormalities often seen in the context of gluten sensitivity. The right scan 2 years later shows considerable progression of the white matter changes. Strict adherence to a GFD is usually associated with no progression of the white matter changes as well as resolution of the headaches. Abbreviations; AGA, antigliadin antibody; GFD, gluten-free diet. *(With permission and courtesy of Professor Marios Hadjivassiliou, Royal Hallamshire Hospital, Sheffield).*[170]

Appendix E1: Studies assessing diagnostic outcomes in self-reported gluten sensitivity [217]

Report	Design	N	Gluten challenge, dosage & duration?	CD diagnostic criteria	Final diagnosis
Kabbani et al (2014)[186]	Retrospective	238	Yes in all cases, dosage not specified, at least 6-8 weeks	Positive TTG/DGP + villous atrophy	42.4% CD*, 52.5% NCGS, 3.8% NCE, 1.3% indeterminate
Aziz et al (2013)[196]	Prospective	200	Yes in HLA-DQ positive, ≥3g of gluten per day for 2 weeks	Positive TTG/EMA + raised IELs to villous atrophy	7% CD, 93% NCGS
Coburn et al (2013)[215]	Retrospective	137	Yes in 56 cases, half declined gluten challenge, dosage and duration not specified	Positive TTG/EMA + villous atrophy	2% CD, 20% LD, 78% NCGS
Kaukinen et al (2000)[216]	Prospective	93	Yes in all cases, dosage not specified, majority at least 1 month	Villous atrophy	9% CD, 8% LD, 20% cereal allergy, ?63% NCGS

TTG, Tissue transglutaminase antibody; EMA, Endomysial antibody; DGP, Deamidated gliadin peptide antibodies; IELs, Intraepithelial lymphocytes; LD, lymphocytic duodenosis; NCGS, Non-coeliac gluten sensitivity; NCE, Non-coeliac enteropathy;* two cases were seronegative CD

Indexed publications

Chapter 1

1. **Aziz I**, Hadjivassiliou M, Sanders DS. Review - The spectrum of noncoeliac gluten sensitivity. *Nature Reviews Gastroenterology & Hepatology. 2015* Sep;12(9):516-26. (Permission granted for non-commercial re-use by Nature Publishing Group)

2. Branchi F*, **Aziz I***, Conte D, Sanders DS. Review - Noncoeliac gluten sensitivity: a diagnostic dilemma. *Current Opinion in Clinical Nutrition and Metabolic Care* 2015 Sep;18(5):508-14. *joint first authors. (Permission granted for non-commercial re-use by Wolters Kluwer Health Lippincott Williams & Wilkins)

3. **Aziz I,** Branchi F, Sanders DS. Review - The rise and fall of gluten! *Proceedings of the Nutrition Society*. 2015 Aug;74(3):221-6. (Permission granted for non-commercial re-use by Cambridge University Press)

4. **Aziz I**, Hadjivassiliou M, Sanders DS. Letter - Self-reported gluten sensitivity; an international concept in need of consensus? *American Journal of Gastroenterology.* 2014 Sep;109(9):1498-9. (Permission granted for non-commercial re-use by Nature Publishing Group)

5. **Aziz I,** Hadjivassiliou M. Editorial - Noncoeliac gluten sensitivity: food for thought. *Nature Reviews Gastroenterology & Hepatology*. 2014 Jul;11(7):398-9. (Permission granted for non-commercial re-use by Nature Publishing Group)

6. **Aziz I**, Hadjivassiliou M, Sanders DS. Editorial - Non-coeliac gluten sensitivity: a disease of the mind or gut? *Alimentary Pharmacology & Therapeutics.* 2014 Jul;40(1):113-4. (Permission granted for non-commercial re-use by John Wiley & Sons)

7. **Aziz I**, Sanders DS. Editorial - Patients who avoid wheat and gluten: is that health or lifestyle? *Digestive Diseases and Sciences*. 2014 Jun;59(6):1080-2. (Permission granted for non-commercial re-use by Springer Link)

8. Mooney PD, **Aziz I**, Sanders DS. Review - Non-celiac gluten sensitivity: clinical relevance and recommendations for future research. *Neurogastroenterology and Motility.* 2013 Nov;25(11):864-71. (Permission granted for non-commercial re-use by John Wiley & Sons)

9. **Aziz I**, Hadjivassiliou M, Sanders DS. Review - Does gluten sensitivity in the absence of coeliac disease exist? *British Medical Journal.* 2012; 345:e7907. (Permission granted for non-commercial re-use by BMJ Publishing Group)

10. Sanders DS**, Aziz I.** Editorial - Non-celiac wheat sensitivity: separating the wheat from the chat! *American Journal of Gastroenterology*. 2012 Dec;107(12):1908-12. (Permission granted for non-commercial re-use by Nature Publishing Group)

11. **Aziz I,** Sanders DS. Review - The irritable bowel syndrome-celiac disease connection. *Gastrointestinal Endoscopy Clinics of North America.* 2012 Oct;22(4):623-37. (Permission granted for non-commercial re-use by Elsevier)

12. **Aziz I**, Sanders DS. Review - Emerging concepts: from coeliac disease to non-coeliac gluten sensitivity. *Proceedings of the Nutrition Society.* 2012 Sep 6:1-5. (Permission granted for non-commercial re-use by Cambridge University Press)

Chapter 3

13. **Aziz I**, Karajeh MA, Zilkha J, Tubman E, Fowles C, Sanders DS. Original article - Change in awareness of gluten-related disorders among chefs and the general public in the UK: a 10-year follow-up study. *European Journal of Gastroenterology & Hepatology.* 2014 Nov;26(11):1228-33. (Permission granted for non-commercial re-use by Wolters Kluwer Health Lippincott Williams & Wilkins)

Chapter 4

14. **Aziz I**, Lewis NR, Hadjivassiliou M, Winfield SN, Rugg N, Kelsall A, Newrick L, Sanders DS. Original article - A United Kingdom study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. *European Journal of Gastroenterology & Hepatology.* 2014 Jan;26(1):33-9.

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Chapter 5

15. **Aziz I**, Branchi F, Pearson K, Priest J, Sanders DS. Original article - A study evaluating the bidirectional relationship between inflammatory bowel disease and self-reported non-celiac gluten sensitivity. *Inflammatory Bowel Diseases.* 2015 Apr;21(4):847-53. (Permission granted for non-commercial re-use by Wolters Kluwer Health Lippincott Williams & Wilkins).

Chapter 6

16. **Aziz I**, Trott N, Briggs R, North JR, Hadjivassiliou M, Sanders DS. Original article - Efficacy of a Gluten-Free Diet in Subjects With Irritable Bowel Syndrome-Diarrhea Unaware of Their HLA-DQ2/8 Genotype. Clin Gastroenterol Hepatol. 2016 May;14(5):696-703

Non-medline articles

1. **Aziz I**, Sanders DS. Non-coeliac gluten sensitivity. *Sugar Nutrition UK* 2015
2. **Aziz I**, Sanders DS. Non-coeliac gluten sensitivity. *GP Pulse* 2014
3. **Aziz I**, Sanders DS. Adult coeliac disease. *Complete Nutrition* 2012
4. **Aziz I**, Briggs R, Sanders DS. Gluten sensitivity in Irritable Bowel Syndrome. *Complete Nutrition* 2012

Book chapters

1. **Aziz I**, Sanders DS. Coeliac disease and nutrition. *Advanced Nutrition and Dietetics in Gastroenterology (Wiley)* 2014.

Abstract presentations at national conferences

Chapter 3

1. Change in awareness of gluten-related disorders among chefs and the general public in the UK: a 10-year follow-up study. *Coeliac Falk Symposium, Amsterdam 2014; United European Gastroenterology Week, Vienna 2014; British Society of Gastroenterology, Manchester 2014 (POSTERS)*

Chapter 4

2. A United Kingdom study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. *British Society of Gastroenterology, Glasgow 2013 (POSTER); United European Gastroenterology Week, Berlin 2013 (ORAL)*

Chapter 5

3. Non-coeliac gluten sensitivity can be present in inflammatory bowel disease, not just irritable bowel syndrome. *Coeliac Falk Symposium, Amsterdam 2014; British Society of Gastroenterology, Manchester 2014 (POSTERS)*

Chapter 6

4. A gluten-free diet is a viable treatment option for the management of diarrhoea-predominant irritable bowel syndrome. *Functional Gastrointestinal Day, Leeds 2015 (ORAL)*

5. A single-blind study evaluating the effects of a gluten-free diet in IBS-Diarrhoea. *International Coeliac Disease Symposium, Prague 2015 (ORAL)*; *Digestive Diseases Federation, London 2015 (ORAL)*; *United European Gastroenterology Week, Barcelona 2015 (ABSTRACT IN THE SPOTLIGHT-ORAL)*

Invited lectures

1. The European Society for Clinical Nutrition and Metabolism, Lisbon 2015 “Non-coeliac gluten sensitivity”

2. Royal Society of Medicine, London 2015 “Non-coeliac gluten sensitivity; an emerging entity”

3. Digestive Disease Federation, London 2015. “Is it non-coeliac gluten sensitivity?”

4. University of Salford, Manchester 2015. “Non-coeliac gluten sensitivity”

5. Academy of Paediatric Gastroenterology, London 2014; “Gluten sensitivity in adults”

6. The Allergy Show, London 2014. “Coeliac disease, non-coeliac gluten sensitivity & irritable bowel syndrome”

7. Food Allergy & Intolerance Specialist Group, London 2014. “How seriously should we take non-coeliac gluten sensitivity?”

8. Coeliac UK, London 2014. “Coeliac disease and non-coeliac gluten sensitivity.”

9. Academy of Paediatric Gastroenterology, University College London 2013. “Non-coeliac gluten sensitivity”

10. Nutrition and Health Live, London 2012. “Gluten sensitivity: Fact or fallacy?”

11. National Primary Care Meeting, Birmingham 2012. “Coeliac disease: getting the diagnosis right”

12. National Centre of Rehabilitation & Education, Derby 2012. “Irritable bowel syndrome and differential diagnosis”

Prizes

Chapter 3

1. Change in awareness of gluten-related disorders among chefs and the general public in the UK: a 10-year follow-up study. *Poster of distinction at Coeliac Falk Symposium, Amsterdam 2014*

Chapter 4

2. The population prevalence of gluten sensitivity and the diagnostic yield in secondary care. *First prize at the 3rd year oral Postgraduate Research Presentations, University of Sheffield 2014*

3. The population prevalence of gluten sensitivity and the diagnostic yield in secondary care. *First prize for oral presentation at the Professor Bardhan Fellowship, South Yorkshire 2013*

4. The population prevalence of gluten sensitivity and the diagnostic yield in secondary care. *Poster of distinction at British Society of Gastroenterology, Glasgow 2013*

Chapter 5

5. Non-coeliac gluten sensitivity can be present in inflammatory bowel disease, not just irritable bowel syndrome. *Poster of distinction at Coeliac Falk Symposium, Amsterdam 2014*

Chapter 6

6. A gluten-free diet is a viable treatment option for the management of diarrhoea-predominant irritable bowel syndrome. *Awarded best young investigator at Functional Gastrointestinal Day, Leeds 2015*

7. Evaluating the efficacy of a gluten-free diet in irritable bowel syndrome-diarrhoea subjects blinded to HLA-DQ genotype status. *Awarded first prize in oral poster presentation at United European Gastroenterology Week, Barcelona 2015*

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