The Neurology of Gluten Sensitivity

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ABSTRACT

Classical coeliac disease (CD) is a well-defined syndrome of small bowel villous atrophy associated with abdominal pain, malabsorption, and weight loss as a result of gluten-sensitivity, reversed rapidly by gluten exclusion diet. Disease associations include dermatitis herpetiformis (DH), Addison's disease, type 1 diabetes mellitus, autoimmune thyroid disease and a variety of neurological disorders. This thesis aims to investigate the hypothesis of the existence of a gluten sensitive neurological disease

CD with coexistent neurological dysfunction is only rarely reported in a neurological setting. 23 cases were reported from the British Neurological Surveillance Unit (BNSU) over 24 months and 13 locally over 31 months. 18 sets of notes (50%) were reviewed. These patients comprise a heterogeneous group of neurological disorders including epilepsy, myelopathy, axonal neuropathy and migraine.

Neurological disorders in patients with confirmed gluten sensitivity may occur simply by chance. In a cohort of 801 CD patients, 54 neurological disorders were identified in 177 patients including stroke (2.9%), migraine (2.7%), epilepsy (2.6%) and carpal tunnel syndrome (2.0%).

More detailed investigation of 35 patients with DH and 53 patients with CD, confirmed a low prevalence of idiopathic neurological abnormalities (DH 11%; CD 25%). Analysis of sera from these patients did not identify the presence of

anti-neuronal antibodies. A novel anti-spinal antibody was identified in over 50% of the subjects with DH but this requires further characterisation.

It has been postulated that patients with idiopathic neurological disease and anti-gliadin antibody (AGA) seropositivity are gluten sensitive. However, AGA lacks disease specificity being found in 10% of healthy blood donors. Screening of 49 unselected multiple sclerosis cases found IgG AGA in 12% of patients and 13% of blood donors confirming that AGA (especially IgG isotype) can be a non-specific finding.

AGA, other food antibodies and tissue transglutaminase antibody (TTG) were measured in patients with idiopathic ataxia (20), hereditary ataxia (7) and idiopathic peripheral neuropathy (32). None of the cases was positive for IgA TTG making occult CD unlikely. Cerebellar ataxia with positive AGA (so-called ‘gluten ataxia’) was rare (4 cases in 2 years from a population of 2 million). All food antibodies tested (AGA, hen’s egg albumen, and cow’s milk lactoglobulin), particularly IgG, were a common finding in both ataxia and peripheral neuropathy groups.

This study found no evidence for gluten neurotoxicity. Serological tests, particularly AGA, need to be interpreted with caution. Further study is required regarding the nature of the association between neurological illness and gluten sensitivity.

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PUBLICATIONS

Papers

Neurological complications of coeliac disease
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Neurological associations of coeliac disease
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Multiple Sclerosis and Occult Gluten Sensitivity
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RJ Lock, DSNA Pengiran Tengah, DJ Unsworth, JJ Ward, AJ Wills. *JNNP* 2005; 76: 1601-1603

Book chapter

“Neurological complications of coeliac disease”
DSNA Pengiran Tengah, AJ Wills, GKT Holmes.
In Mayberry J editor. Gastroenterology Update. Oxford: Radcliffe Medical Press; 2004.

Presentations

Oral

Gluten sensitivity and neurological illness

South West of England Neurological Association (SWENA) Meeting, 18th June 2004

Poster

Dermatitis herpetiformis, coeliac disease and neurological dysfunction

World Congress of Neurology, London, 18th-22nd June 2001

Dermatitis herpetiformis, coeliac disease and neurological dysfunction

Association of British Neurologists Autumn Meeting, Durham, 12th-14th September 2001

Multiple sclerosis and occult gluten sensitivity

Association of British Neurologists Autumn Meeting, London, 2nd-4th October 2002

An anti-spinal antibody in dermatitis herpetiformis patients

Association of British Neurologists Joint Congress with Neurological Association of South Africa, Capetown , 29th January to 1st February 2003

Features of patients with coeliac disease and coexistent neurological illness: a retrospective study.

Association of British Neurologists Spring Meeting, Cardiff, 2nd-4th April 2003

LIST OF ABBREVIATIONS

CD	Coeliac disease
DH	Dermatitis herpetiformis
GFD	Gluten free diet
HLA	Human leukocyte antigen
ARA	Antireticulin antibody
AGA	Antigliadin antibody
EMA	Endomysial antibody
AJA	Antijejunal antibody
TTG	Tissue transglutaminase antibody
IEL	Intra-epithelial lymphocytes
EATCL	Enteropathy-Associated T-cell Lymphoma
BNSU	British Neurological Surveillance Unit
GPRD	General Practice Research Database
CSF	Cerebrospinal fluid
MS	Multiple sclerosis

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Chapter 1 – Introduction

1.1 Aims

This thesis aims to further elucidate the association between gluten sensitivity and neurological disorders. This will be achieved by the following means:

1. Summary of the literature on this topic in terms of epidemiology, pathogenesis and complications of known gluten sensitive disease namely coeliac disease (CD) and dermatitis herpetiformis (DH), as well as review of the evidence for the existence of gluten sensitive neurological syndromes
2. Detailed case report analysis of patients with co-existent neurological dysfunction and CD
3. Study of the features and prevalence of neurological abnormalities in patients with confirmed gluten sensitivity (CD and DH)
4. Study of the prevalence of gluten sensitivity in patients with specific neurological abnormalities namely idiopathic peripheral neuropathy, idiopathic ataxia and multiple sclerosis
5. Immune profiles and genetic analysis of patients with neurological dysfunction and CD.

1.2 Background - Coeliac disease

Dicke (Dicke 1950) first demonstrated the harmful effect of gluten ingestion in patients with coeliac disease (CD). Classically CD is known to be a chronic

inflammatory disease of the small bowel mucosa associated with malabsorption, steatorrhoea and weight loss as a result of sensitivity to gluten. Gluten is found in foods containing wheat, barley and rye. The treatment i.e. strict gluten-free diet (GFD) results not only in symptomatic improvement but also restoration of normal mucosal architecture. Furthermore, re-introduction of gluten results in symptomatic and histological relapse. Although biopsy of small bowel remains the diagnostic “gold standard” for CD, antibody testing can be a useful supplementary investigation.

1.3 Epidemiology

Ideas about the incidence and prevalence of CD have changed greatly. For reasons which are not completely understood, adult incidence rates have increased (Jenkins, Hawkes et al. 1998; Hawkes, Swift et al. 2000). Furthermore, population screening studies have shown that CD remains underdiagnosed in adults (Unsworth and Brown 1994; Hin, Bird et al. 1999). CD is mainly found in Western European populations and countries where Europeans have migrated. Prevalence rates estimated from population screening are as high as 1:82 in adults in New Zealand (Cook, Burt et al. 2000), and 1.3% in healthy Swedish children (Carlsson, Axelsson et al. 2001). The variable prevalence rates amongst different populations may be explained by genetic factors. However, interpretation of epidemiological data is complicated because of variations in disease definition and case ascertainment. One study has shown a three-fold increase in diagnosis rate in a hospital with a special interest compared to two other hospitals (Swinson and Levi 1980).

CD was once thought to be a childhood illness typically presenting with malnutrition and abdominal symptoms but is now recognised as a condition affecting all ages that can also present with atypical and often subtle symptoms. Reasons proposed for this have included altered dietary habits such as increased breast-feeding and longer time to weaning to solids (Ivarsson, Hernell et al. 2002). Most adult patients have mild symptoms that on direct questioning would be compatible with a diagnosis of CD. They may present with non-specific or trivial complaints and the diagnosis is only suspected from abnormalities found in routine blood tests such as anaemia or from the results of specific serological tests.

1.3.1 Changing pattern of coeliac disease-The Coeliac Iceberg

Some asymptomatic patients with an enteropathy characteristic of CD are labelled “silent CD” while other patients who have an apparently normal small bowel biopsy but develop typical histological features later in life are regarded as having “latent CD”. These observations have led to the concept of a “coeliac iceberg” made up of a visible part of those who are diagnosed clinically and a far larger submerged portion that includes all individuals who are undiagnosed because of asymptomatic, occult or latent disease (Catassi, Ratsch et al. 1994). This rise in diagnosis rate may not represent actual change in incidence because the advent of serological markers has changed our diagnostic ability, resulting in CD becoming more commonly recognised. The implications of this improved diagnostic rate, particularly in the less severely affected, remain unclear.

1.4 Pathogenesis

The pathogenesis of CD as an autoimmune condition is multifactorial with a combination of genetic predisposition and environmental influences resulting in tissue injury caused by auto-reactive T cells or antibodies. This process is not completely understood but current evidence suggests that the disease is triggered by an external antigen, gluten, in a genetically susceptible individual. The disease appears to be specific to humans and thus satisfactory animal models have made pathophysiological research difficult.

1.4.1 Genetic factors

A major genetic role is implied by the occurrence of multiple cases within the same family (Auricchio, Greco et al. 1988) with studies of first degree relatives having as many as 10% with the disease (Hogberg, Falth-Magnusson et al. 2003). Twin concordance is as high as 75% in monozygotic twins (Greco, Romino et al. 2002). However the 25% discordance in monozygotic twins implies a significant contribution by environmental factors.

The main component of genetic predisposition lies in the major histocompatibility complex (MHC), a collection of genes on chromosome 6 that encodes the human leucocyte antigens (HLA). HLA are cell surface glycoproteins (found on the surface of B cells, monocytes, activated T cells and enterocytes) that bind short peptides degraded or generated by the cell and present these antigens to CD4+ T helper cells. The role of MHC through molecular mimicry has been proposed as a possible mechanism for development of autoimmune disease that combines the influence of genetics and the environment. Alternatively, the HLA DQ molecule may select in the thymus for specific T cell receptors involved in the pathogenesis of CD.

Gliadin-specific T-cells in the small intenstines of coeliac patients use HLA DQ2 in antigen recognition to mount an inflammatory response (Lundin 2002).

HLA Class II molecules are estimated to account for approximately 40% of the genetic load (Bevan, Popat et al. 1999). Greco *et* al (Greco, Romino et al. 2002) showed substantial evidence that the very strong genetic component in CD is only partially due to the HLA region by looking at the genetic load in CD and estimating the concordance rate for the disease among twin pairs in a large population based study adjusting for HLA haplotype.

More than 95% of patients with defined gluten sensitive disease i.e. CD and DH, carry the HLA DQ2 or DQ8 haplotype (Zubillaga, Vidales et al. 2002). The prevalence of these HLA haplotypes varies enormously in different ethnic groups but in the UK population is estimated at 40%. They are therefore common haplotypes in Caucasian populations and other factors must exist for development of gluten sensitivity as the majority of people with this haplotype will not develop CD despite gluten ingestion.

1.4.2 Environmental factors

CD is exacerbated by ingestion of foods containing wheat, rye, and barley. Toxic fragments in these cereals are rich in proline and glutamine and are collectively known as prolamins. The active prolamin fractions in each of these cereals are gliadin in wheat, secalin in rye and hordein in barley. In oats the prolamin fraction, avenin, is small, only 5-15%, which may account for tolerance of oats in the diets of some CD sufferers. Studies have shown that oats are probably not harmful and therefore an acceptable alternative in a GFD (Janatuinen, Kemppainen et al. 2002).

Most studies have focussed on wheat due to its nutritional importance. Fractionation of wheat produces a starch fraction and a protein fraction, gluten, of which gliadin is the soluble component. Gliadin accounts for 50% of wheat protein and within a single wheat fraction, there are approximately 45 gliadins which are subdivided into α, β, γ and ω subfractions depending on their electrophoretic mobility. The amino acid sequence has been determined in the A-gliadin molecule and a number of *in vitro* studies and *in vivo* studies have indicated the presence of one or more CD activating epitopes on this molecule (Ciclitira 2003). A recent study showed that a peptide corresponding to residues 56-75 of α-gliadin exacerbates CD *in vivo* (Fraser, Engel et al. 2003).

It has been suggested that infection with adenovirus 12 sensitises the genetically predisposed host to A-gliadin by molecular mimicry resulting in gluten sensitive enteropathy (Kagnoff, Paterson et al. 1987). The E1b protein of adenovirus 12 has been reported to share a similar amino acid sequence to A-gliadin.

1.4.3 Immunological factors

The intestinal response in CD is triggered by gluten resulting in activation of macrophages, plasma cell recruitment, release of inflammatory cytokines and CD8 T lymphocyte infiltration. Although plasma cells produce antibodies to gliadin and connective tissue autoantigens, the intestinal damage is a cellular rather than humoral process. Indeed, Webster and co-authors showed that CD occurred in a hypogammaglobulinaemic patient suggesting that these

antibodies are not crucial to pathogenesis (Webster, Slavin et al. 1981). They were unable to demonstrate specific cellular immunity to a subfraction of gluten implying that immunological reactions have a much less clear role in the pathogenesis of CD than previously thought.

1.5 Recognised associations of coeliac disease

Gluten intolerance can be considered a spectrum of conditions ranging from frank CD to latent CD and including extra-intestinal gluten intolerance and non-coeliac gastrointestinal gluten intolerance such as that seen in irritable bowel syndrome. Patients with a genetic susceptibility to gluten may have no intestinal abnormalities on small bowel biopsy. The precise mechanism for the activation of gut inflammation by gluten is not known although it is presumed to be immunological. Immune mechanisms such as the deposition of circulating immune complexes in other organs are also thought to cause the extra-intestinal manifestations of gluten sensitivity.

Dermatitis herpetiformis (DH), characterised by IgA deposition in the papillary dermis, is a blistering skin condition (Figure 1.1) that exemplifies an extra-intestinal manifestation of gluten sensitivity. The “gold standard” for diagnosis of DH remains skin biopsy. There is immuno-pathological and genetic data that DH and CD are closely related conditions on the basis of treatment response, common HLA, twin studies and autoantibody profiles. CD and DH share the same strong association with the HLA DQ2 haplotype, presence of characteristic small intestinal changes and a similar rate of disease associations e.g. Type 1 Diabetes Mellitus.

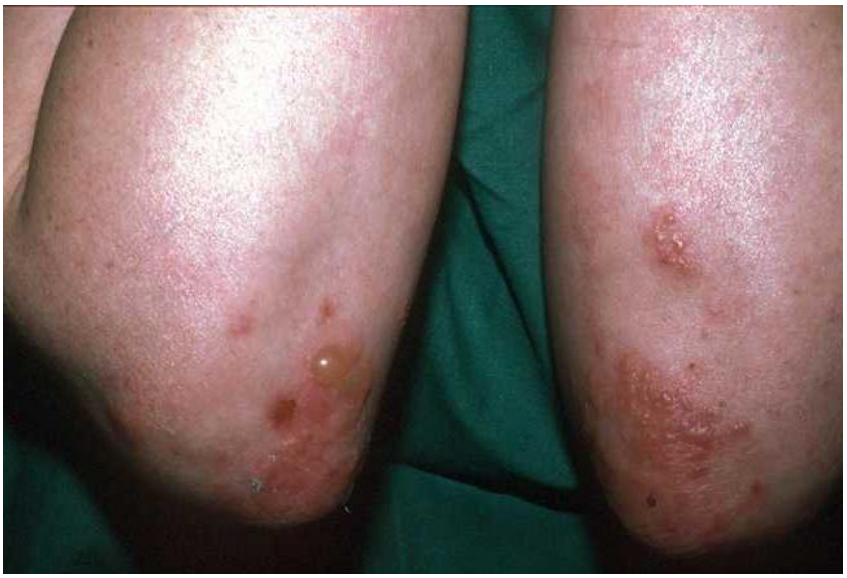


Figure 1.1 Blisters on the elbows of a patient with dermatitis herpetiformis

It has been suggested that CD is associated with conditions such as Addison's disease (O'Leary, Walsh et al. 2002), Type I Diabetes Mellitus (Holmes 2001) and autoimmune thyroid disease (Sategna-Guidetti, Volta et al. 2001). A similar association has been proposed with other autoimmune conditions such as primary biliary cirrhosis (Sorensen, Thulstrup et al. 1999) and Sjogren's syndrome (Iltanen, Collin et al. 1999).

The association of CD with a variety of neurological disorders has also been frequently described but whether a specific neurological association occurs with CD remains unproven. The basis of these associations is unclear. A genetic mechanism has been postulated. However, as CD is relatively common these may be chance findings. This is discussed in more detail in Chapter 2.

1.6 Diagnosis

1.6.1 Serological testing

Improved screening for antibodies associated with CD i.e. antireticulin antibodies (ARA), IgA and IgG antigliadin antibodies (AGA), endomysial antibodies (EMA), anti-jejunal antibody (AJA) and tissue transglutaminase antibodies (TTG), has dramatically increased the numbers of diagnoses of CD in recent years. However, these antibodies have varying specificities and sensitivities, particularly when applied to population screening, and it is generally recognised that IgA ARA, AEA and TTG have heightened specificity when compared to AGA (Lagerqvist, Ivarsson et al. 2001).

The presence of positive coeliac antibodies with normal small bowel architecture remains problematic. Follow-up of patients with normal small bowel architecture and positive coeliac antibodies has shown that positive ARA is a good predictor for later development of the disease when compared to AGA particularly the IgG subclass of AGA (Collin, Helin et al. 1993). In GSE the positive predictive value of isolated IgG AGA positivity and an initially normal small bowel biopsy is 0 %. In a further study the positive predictive value of EMA was 100% compared to only 28% for IgA AGA (Valdimarsson, Franzen et al. 1996).

The available evidence from absorption and other studies suggests that ARA, EMA, AJA and TTG are one and the same. All four are believed to be antibodies to the same enzyme, tissue trans-glutaminase, simply giving different appearances when tested on different tissue substrates e.g. monkey

oesophagus and human foetal jejunum (AEA and AJA respectively). These autoantibodies are unique because they are predictably invoked in susceptible patients (CD) by an environmental agent, namely gluten, disappearing within several months of a strict GFD and reappearing following gluten challenge. AGA on the other hand is an anti-wheat protein antibody, which, like other simple food antibodies e.g. anti-ovalbumin and anti-casein, is extremely disease non-specific, particularly when of IgG isotype.

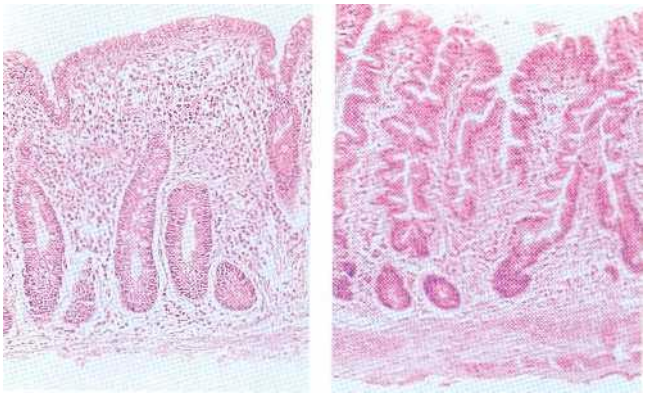
1.6.2 Small bowel biopsy

Small bowel biopsy is the only definitive test for CD and shows characteristic subtotal or total villous atrophy and crypt hypertrophy (Figure 1.2). There is evidence of an inflammatory cell infiltrate in the mucosa which in the epithelium consists almost exclusively of intra-epithelial lymphocytes (IEL) and in the lamina propria consists of plasma cells, lymphocytes and macrophages. There are degrees of severity of small intestinal lesions ranging from a structurally normal mucosa with increased density of IELs expressing γ/δ T cell receptors (Grade 1) to irreversible atrophy seen in jejunoileitis and 'Enteropathy-Associated T-cell Lymphoma (EATCL).

The Marsh classification is used to describe the range of abnormalities seen in the mucosa of patients with CD (Marsh 1992). Marsh Type I lesions have normal mucosal architecture with a lymphocytic infiltration of the villous epithelial layer. An IEL count over 30-40 per 100 enterocytes is arbitrarily used as a threshold denoting a significant increase. In a Marsh Type II lesion, in addition to these findings, crypt hyperplasia and increased mitotic activity can be seen. Marsh Type III lesions are characterised by villous atrophy

ranging from partial (IIIA), subtotal (IIIB) and total (IIIC) as described by Oberhuber and colleagues (Oberhuber, Granditsch et al. 1999). Marsh type IV lesions are rare and are seen in refractory CD.

Whilst recognising that patients with so-called 'latent CD' do exist, this is only rarely described and even in DH where 90% of patients do not have gastrointestinal symptoms, they are all said to have gluten-sensitive enteropathy (Reunala 1998). A flat small bowel mucosa can occur in numerous other conditions such as gastroenteritis, tropical sprue, cow's milk intolerance and immunodeficiency syndromes.



(a) (b)

Figure 1.2 Appearance of small bowel mucosa using light microscopy (a) flattened mucosa in a patient with untreated coeliac disease. (b) normal mucosa showing digitate villi

1.7 Complications

Reported complications of CD include malignancy, ulcerative jejunitis, disorders of bone metabolism, reduced fertility and splenic atrophy. The overall increased mortality in CD is estimated at twice that of the general

population (Logan, Rifkind et al. 1989) and this is mainly attributable to malignancy. Besides lymphoma patients are also at greater risk of oesophageal and small intestinal carcinoma. One study by Holmes *et al* (Holmes, Prior et al. 1989) found a two-fold increase of CD patients in developing cancer. There is evidence to suggest that overall cancer risk reduces to near normal after 5 years on a GFD hence the emphasis on early diagnosis and treatment. A recent review suggests that the magnitude of the incidence of malignant complications was over-estimated although there is a definite increased risk of Non-Hodgkins lymphoma of any site, particularly EATCL (Catassi, Bearzi et al. 2005).

1.8 Treatment

The mainstay of treatment of CD is lifelong exclusion of gluten-containing cereals in the diet. Appropriate dietary counselling is essential particularly if gluten-rich products (bread and pasta) are significant dietary staples. Furthermore, patients need to be warned regarding foods in which gluten may not be an obvious constituent e.g. sauces ('hidden gluten'), inadequately labelled food and food contamination. Compliance is extremely variable with study figures ranging from 45 to 94%. There are many reasons for this including cost (although in Britain, CD patients should receive staple gluten free products on prescription), restrictions on lifestyle, peer pressure particularly amongst adolescents and finally, non-perceived benefit in patients with silent disease. Serum AGA is a useful measure of compliance as this reduces to normal within a few months of GFD.

The majority of paediatric patients respond well to a GFD whereas 10-15% of adult patients do not. There is a role for restricting either dairy products or other food products for a short period. There may be ongoing symptoms from severe damage of small intestinal enterocytes resulting in disaccharide deficiency for example. In a small minority of patients, immunosuppressants may become necessary such as steroids or even cyclophosphamide and azathioprine.

It is recommended that patients with CD should have lifelong medical follow-up to assess weight, routine blood indices and to address any health concerns that may arise particularly any new symptoms, diarrhoea or weight loss that may suggest a malignant complication.

1.9 Discussion

There is greater recognition that CD is increasingly prevalent worldwide largely as a result of improved serological screening and the widespread use of fibreoptic endoscopy to obtain small bowel biopsies. Although there are numerous well-recognised manifestations of CD, gastrointestinal features are the best recognised. Numerous other associations have been frequently described including neurological disorders, Addison's disease and hypothyroidism, however the association of CD with these conditions is unclear. Whether a specific neurological complication occurs in CD, apart from those secondary to malabsorption, remains unproven. It has been suggested that patients with a variety of idiopathic neurological conditions should be screened for CD. Among this group even those patients without

overt CD may have positive antigliadin antibodies (AGA) and HLA profile characteristic of CD (Hadjivassiliou, Grunewald et al. 1998).

The definition of disease mechanisms of complex inflammatory disorders is difficult with extensive interactions between genetic and environmental factors. CD, the prototypical disease of gluten sensitivity, is no exception. However with CD, key genetic and environmental factors have been identified. We have some understanding of predisposing HLA molecules, antigenic epitopes and disease-relevant T-lymphocytes. The main aim of this thesis was to gain a further understanding of the association of CD and neurological disease. CD is still not fully understood and research continues into its genetics, pathogenesis, epidemiology and immunology amongst others. Such comprehensive insight was clearly not within the constraints of this thesis but I will attempt to address some important misconceptions with regards to spectrum of neurological association, prevalence and serological screening.

Chapter 2 – Neurological associations of Coeliac Disease

2.1 Aim

This chapter aims to summarise the known literature on neurological associations of CD including possible aetiology, pathological findings and treatment potential. This is a comprehensive literature review performed by searching PubMed for articles written up to the end of 2006.

2.2 Introduction

The putative association of CD with neurological disorders has attracted much interest in recent years, the most frequent being epilepsy, cerebellar ataxia, peripheral neuropathy, dementia and depression. However the nature and mechanism of this association remains unclear.

The first detailed descriptive study of patients with CD (confirmed on jejunal biopsy) and a neurological deficit was published by Cooke and Smith in 1966 (Cooke and Smith 1966). They described a group of 16 patients with a variety of neurological findings. The predominant abnormality was a sensory ataxia suggesting damage to the dorsal columns in the spinal cord. These patients deteriorated despite dietary gluten restriction. Three patients had evidence of cerebellar dysfunction. Post-mortem study of 9 of these patients demonstrated spongiform changes in the lateral and posterior columns.

Since then many different neurological disorders have been described in association with CD ranging from the relatively common like epilepsy, neuropathy and migraine to rarer associations such as chorea (Pereira, Edwards et al. 2004) and leukoencephalopathy (Beyenburg, Scheid et al. 1998).

2.3 Neurological disturbances described with coeliac disease

2.3.1 Epilepsy

An increased prevalence of epilepsy in patients with CD has been proposed (Chapman, Laidlow et al. 1978). In addition, a high prevalence of CD has been seen in patients with bilateral occipital calcification (Ventura, Bouquet et al. 1991; Magaudda, Dalla Bernardina et al. 1993; Lea, Harbord et al. 1995), mainly from Italian centres. Gobbi *et al* (Gobbi, Bouquet et al. 1992) looked at two groups of patients with epilepsy and found a high incidence of CD in patients with unexplained cerebral calcification and epilepsy (24/31) and a high incidence of cerebral calcification in patients with CD and epilepsy (5/12). Low folate levels were also found which might be attributable either to malabsorption or chronic anticonvulsant therapy. Interestingly, cerebral calcifications have previously been reported in other folate deficiency states (Kay, Knapton et al. 1972; Flament-Durand, Ketelbant-Balasse et al. 1975).

This association of intracerebral calcification with epilepsy and CD has not been demonstrated in an Irish study (Cronin, Jackson et al. 1998). Although CD was found to occur with increased frequency in patients with epilepsy (1/44), no patient had cerebral calcification on CT scanning suggesting that cerebral calcification is not a feature of epileptic patients screened at that hospital. This suggests that this may be an ethnic or geographic finding, perhaps confined to Italian populations (Figure 2.1). Many of these studies are case reports or small case series, and in addition are flawed by lack of blinding.

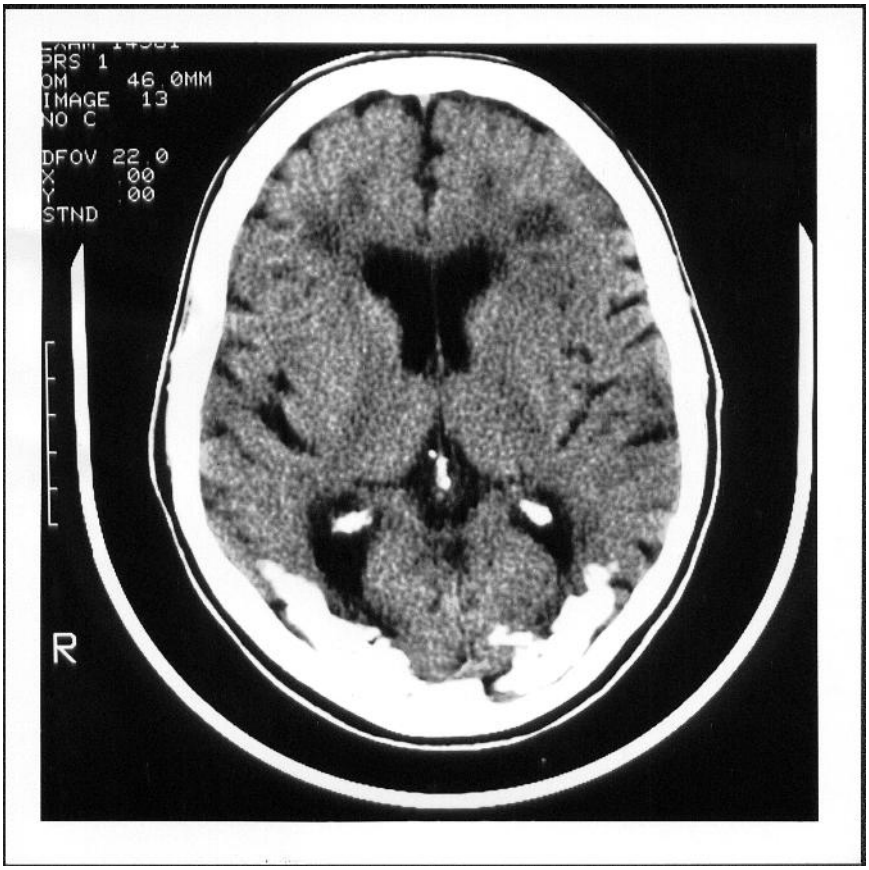


Figure 2.1 Computed tomography of the brain showing bilateral occipital calcifications in a patient with coeliac disease and epilepsy [photo courtesy of Dr Gordon Plant, The National Hospital for Neurology and Neurosurgery, Queen Square]

2.3.2 Cerebellar ataxia

Spinocerebellar and cerebellar disorders have been described by a number of researchers. There have been frequent reports of ataxia in classic CD where gastro-intestinal symptoms were concomitant or preceded the neurological complications (Ghezzi, Filippi et al. 1997). There are also numerous case reports of a cerebellar syndrome where CD was a subsequent diagnosis (Finelli, McEntee et al. 1980; Ward, Murphy et al. 1985; Beversdorf, Moses et al. 1996). Myoclonic ataxia (Ramsay-Hunt Syndrome) may be associated with CD (Lu, Thompson et al. 1986; Bhatia, Brown et al. 1995; Chinnery, Reading et al. 1997; Smith, Saldanha et al. 1997). AGA positivity has been demonstrated in the cerebrospinal fluid (CSF) as well as the serum of a single

patient with myoclonic ataxia possibly representing leakage through a damaged blood brain barrier (Chinnery, Reading et al. 1997).

The nature of the association between idiopathic cerebellar ataxia and occult gluten sensitivity is controversial. An increased frequency of AGA positivity has been reported in patients with idiopathic ataxia (Hadjivassiliou, Grunewald et al. 1998; Pellecchia, Scala et al. 1999; Hadjivassiliou, Grunewald et al. 2003) compared to control groups of ataxia of known aetiology. However, other researchers have not replicated these findings. 32 adult patients with idiopathic cerebellar ataxia were screened using AGA (IgA/IgG), ARA, EMA and TTG (Combarros, Infante et al. 2000). None of these patients were positive for any of these antibodies suggesting no association between these two conditions. Interestingly, Bushara and co-workers (Bushara, Goebel et al. 2001) found a high prevalence of AGA in patients with sporadic ataxias (7/26) as well as hereditary ataxias (9/24) highlighting the poor specificity of AGA as a screening tool for gluten sensitivity. This has been confirmed by further studies in other neurological disorders such as Huntington's disease (Bushara, Nance et al. 2004) and multiple sclerosis (Pengiran Tengah, Lock et al. 2004) (the latter is discussed in more detail in Chapters 7 and 8).

These studies are relatively small and are flawed by lack of blinding and a reliance on AGA testing. The key published studies are summarised in

		Type of study	**Number of patients**	**Key findings**
Hadjivassiliou, Grunewald et al	1998	Cohort study	28 ataxia patients screened positive for AGA	19 patients with peripheral neuropathy on nerve conduction studies
Pellecchia,	1999	Case	24 idiopathic	3/24 idiopathic

Scala et al		control study	ataxia 23 symptomatic ataxia	ataxia found positive only in the idiopathic group
Combarros, Infante et al	2000	Cohort study	32 idiopathic ataxia	No antibody association
Bushara, Goebel et al	2001	Case control study	26 sporadic ataxia 24 hereditary ataxia	High prevalence of AGA in both hereditary and sporadic ataxia
Hadjivassiliou, Grunewald et al	2003	Case control study	284 ataxia:- 59 familial 132 sporadic ataxia 33 Multiple systems atrophy	Higher prevalence of AGA in sporadic group (statistically significant)

Table 2.1 Table summarizing key findings in published studies of cerebellar ataxia and gluten sensitivity

2.3.3 Peripheral Neuropathy

Peripheral neuropathy in patients with established CD has been observed by a number of researchers (Binder, Solitare et al. 1967; Kaplan, Pack et al. 1988; Simonati, Battistella et al. 1998) (Chin, Sander et al. 2003). The neuropathy may be of axonal or demyelinating type. An association with antiganglioside antibody has been described (Alaedini, Green et al. 2002).

In two studies, the neuropathy appeared to occur because of non-compliance with a GFD and improved on reintroduction of a GFD (Kaplan, Pack et al. 1988; Polizzi, Finocchiaro et al. 2000). However there is little evidence of a response to a GFD in other studies. These are all case reports and provide no strong evidence for an association.

2.3.4 Other Neuropsychiatric Associations

Numerous other neurological and psychiatric disorders have been described in association with CD and these include myelopathy, dementia (Collin, Pirttila et al. 1991), brainstem encephalitis (Brucke, Kollegger et al. 1988) chorea (Pereira, Edwards et al. 2004) and chronic progressive leukoencephalopathy (Beyenburg, Scheid et al. 1998). Depression is felt to be a significant complication of CD (Goldberg 1970), and appears to be more severe in unrecognised CD (and thus untreated) or in poorly compliant patients (Hallert, Astrom et al. 1982). It has also been suggested that schizophrenia is associated with CD (Dohan 1966). However, this remains contentious, as other studies have not found an association (Peleg, Ben-Zion et al. 2004). Most of these studies are either case reports or small case series and hence any association is difficult to prove.

2.4 Aetiology

The aetiology of neurological diseases associated with CD remains in doubt. Numerous theories have been put forward including immunological, hereditary and nutritional. Unfortunately, the heterogeneity of associated illnesses makes elucidating a unifying causative effect due to CD extremely difficult.

2.4.1 Role of Nutritional Deficits in the Neurological Complications of Coeliac Disease

Nutritional deficiencies may play a role in development of neurological deficits in untreated CD, because of overt or occult malabsorption. Some of these are briefly discussed below. However, it is now acknowledged that these deficiencies are not sufficient factors as vitamin replacement is rarely helpful (Ward, Murphy et al. 1985; Lu, Thompson et al. 1986; Muller, Donnelly et al. 1996; Simonati, Battistella et al. 1998) and hypovitaminosis is not always

detectable (Kaplan, Pack et al. 1988; Bhatia, Brown et al. 1995). Moreover, very often no neurological abnormality is detectable even in the presence of profound vitamin deficiency (Morris, Ajdukiewicz et al. 1970).

2.4.1.1 Calcium

Hypocalcaemia may present with neuropsychiatric complications including irritability, delirium, psychosis, depression, paraesthesiae around the mouth and distal extremities and tetany. Osteomalacic myopathy may occur with mild hypocalcaemia, affecting proximal muscles and associated with bony pain and tenderness and a raised alkaline phosphatase. Both tetany [(Rubinstein, Liron et al. 1982) and myopathy (Hardoff, Sharf et al. 1980) have been reported in CD patients.

2.4.1.2 Folic Acid

Folic acid deficiency has been particularly implicated in patients with CD and epilepsy. Ventura *et al* (Ventura, Bouquet et al. 1991) described two cases of focal occipital epilepsy with cerebral calcifications and CD. They had previously been poorly responsive to antiepileptics. However, a GFD and folate supplementation resulted in complete EEG normalisation in 1 patient and marked improvement in the other. Gobbi and co-workers (Gobbi, Bouquet et al. 1992) found low folate levels in 17 out of 19 patients with cerebral calcifications, epilepsy and CD, which they attributed to either malabsorption or anticonvulsant therapy. They suggested that low folate levels might be pathological as cerebral calcifications have previously been reported in other folate deficiency states such as in the use of antifolate treatments and radiotherapy in leukaemic children (Kay, Knapton et al. 1972; Flament-Durand, Ketelbant-Balasse et al. 1975). This association was further

highlighted by Bye and co-workers (Bye, Andermann et al. 1993) who described a syndrome of bilateral occipital calcification, epilepsy and folic acid deficiency related to CD. In the two patients with myoclonic ataxia described by Lu (Lu, Thompson et al. 1986), there was subtotal villous atrophy on jejunal biopsy, as well as reduced folate and vitamin E levels. A GFD and vitamin supplementation did not improve the neurological disorder.

2.4.1.3 Pyridoxine (Vitamin B_6)

Pyridoxine deficiency has been demonstrated in patients with CD. The absorption mechanism is complex. With damage to the upper duodenal mucosa, it appears that pyridoxine can be absorbed in the more distal intestine. However, this capacity appears to be rather limited (Reinken and Zieglauer 1978). A study by Morris *et al* (Morris, Ajdukiewicz et al. 1970) looked at the possible relationship between pyridoxine levels and neurological disorder in a group of 30 patients with adult CD. Although pyridoxine levels were lower in patients who were non-compliant with a GFD, there was no apparent relationship between pyridoxine levels and neurological deficit. Hallert and co-workers (Hallert, Astrom et al. 1983) looked at the treatment of depression in patients with CD and found an improvement in depressive symptoms after 3 years of GFD coupled with pyridoxine therapy.

2.4.1.4 Vitamin B_{12}

In a study of 35 newly diagnosed CD patients, vitamin B_{12} deficiency was found in 15 (45%), 3 of who had previously developed cold peripheries and paraesthesiae (Dahele and Ghosh 2001).

2.4.1.5 Vitamin E

Vitamin E is important for maintaining normal neurological function. Furthermore, in other conditions such as abetalipoproteinaemia, where hypovitaminosis E is secondary, vitamin E replacement may lead to improvement (Muller, Lloyd et al. 1983). Vitamin E deficiency has been demonstrated in patients with CD and this is reversible on a GFD (Muller, Harries et al. 1974). There are reports of CD, vitamin E deficiency and cerebellar ataxia with improvement following vitamin E replacement therapy (Mauro, Orsi et al. 1991; Battisti, Dotti et al. 1996; Beversdorf, Moses et al. 1996).

2.4.1.6 Biopterin

Biopterin compounds, namely biopterin, 7,8-dihydrobiopterin, and 5,6,7,8-tetrahydrobiopterin have an important role in the metabolism of various neurotransmitters. Low levels have been demonstrated in patients with CD, and it has been suggested that their deficiency may play a role in the development of neurological syndromes (Cooke 1978). Leeming and Blair studied mean serum biopterin levels and found these to be significantly lower not only in CD but also in senile dementia, lead poisoning, malignant carcinoid (Leeming and Blair 1980).

2.4.1.7 Carnitine

Carnitine is required for muscle energy production and is absorbed via a transport mechanism in the small bowel. A small study by Lerner *et al* (Lerner, Gruener et al. 1993) compared carnitine levels in patients with CD, gastrointestinal disease with a normal small bowel biopsy and normal subjects. Carnitine levels were significantly lower in patients with CD.

2.4.2 Altered Autoimmunity and Inflammatory Disorders

Ghezzi and co-workers (Ghezzi, Filippi et al. 1997) described a man with known CD whose small bowel biopsy revealed ulceration and an inflammatory infiltrate. However, two years after diagnosis, despite a GFD, his gastrointestinal symptoms deteriorated and he was given steroids. His gastrointestinal symptoms improved but he subsequently developed a relapsing-remitting brainstem and cerebellar syndrome. Each relapse was treated with steroids and this appeared to improve his symptoms. Serial MRI scanning during the course of his illness showed multiple enhancing lesions. At the start of his illness, corticosteroid administration improved the appearance of the lesions on MRI. CSF examination was initially normal but with subsequent relapses, the presence of oligoclonal IgG bands and an increased CSF/serum albumin ratio was demonstrated, indicating damage to the blood-brain barrier. However, it could be argued that this patient had concurrent CD and multiple sclerosis.

An immune mediated mechanism was also suggested in a patient with chronic progressive leukoencephalopathy and CD because of a CSF lymphocytosis and elevated serum immunoglobulin levels (Beyenburg, Scheid et al. 1998). A patient with treatment-resistant seizures and CD was described by Rush *et al* (Rush, Inman et al. 1986). He was found to have an isolated CNS vasculitis on brain biopsy. There was clinical and radiographic improvement following treatment with prednisolone and cyclophosphamide.

2.4.3 Gluten neurotoxicity

It has been suggested that gluten is neurotoxic. Dohan (Dohan 1966) proposed that cereals might be a cause of schizophrenia. Hadjivassiliou *et al*

((Hadjivassiliou, Gibson et al. 1996; Hadjivassiliou, Chattopadhyay et al. 1997; Hadjivassiliou, Grunewald et al. 1998; Hadjivassiliou, Grunewald et al. 2001; Hadjivassiliou, Grunewald et al. 2003) and Pellechia *et al* (Pellecchia, Scala et al. 1999) have both claimed that AGA positivity leads to immune mediated neurological damage. However the presence of AGA positivity in other conditions such as genetic ataxias (Bushara, Goebel et al. 2001), Huntington's disease (Bushara, Nance et al. 2004) and multiple sclerosis (Pengiran Tengah, Lock et al. 2004) suggest that AGA positivity may be an epiphenomenon.

Cross-reaction of gluten-related antibodies with nervous system structures has been postulated. Antibodies cross-reacting with Purkinje cells have been observed (Hadjivassiliou, Boscolo et al. 2002). Another study showed reactivity of IgA antibodies (AGA and EMA) with human brain vessel structures from sera of patients with untreated CD but not from sera of CD patients on GFD or non-coeliac controls (Pratesi, Gandolfi et al. 1998). These findings were based on immunohistochemistry and non-specific binding may be an alternative explanation.

2.5 Pathological Findings

There is limited available histological data on patients with CD and neurological complications. There does not appear to be a set of distinctive or unifying histopathological features. Some patients have atrophy, gliosis or inflammatory infiltrates in a variety of combinations (Wills 2000). In the earliest post-mortem data of 9 coeliac patients with neurological disorder the changes

were mainly confined to the spinal cord and consisted spongiform changes in the lateral and posterior columns (Cooke and Smith 1966).

Bye and co-workers (Bye, Andermann et al. 1993) reported the pathological findings of a patient with bilateral occipital calcifications who had a right occipital resection because of intractable epilepsy. The patient was subsequently shown to have folic acid deficiency associated with CD. The excised brain tissue showed patchy pial angiomatosis resembling but not identical to that found in Sturge-Weber syndrome. In addition, there were clusters of calcification, atrophic superficial and intermediate white matter, abundant macrophage deposition and moderate gliosis.

Beyenburg *et al* (Beyenburg, Scheid et al. 1998) described a patient with chronic progressive leukoencephalopathy. Limited data available from a brain biopsy showed a severe reduction in myelinated nerve fibres with no evidence of axonal loss, a significant number of macrophages and gliosis with reactive astrocytes.

Hadjivassiliou and co-workers (Hadjivassiliou, Grunewald et al. 1998) reported post mortem examination on two patients. In the first patient, there was patchy loss of Purkinje cells throughout the cerebellar cortex, astrocytic gliosis, neuropil vacuolation and a diffuse infiltrate with T cells in the cerebellar white matter. Perivascular cuffing with inflammatory cells, mainly T-cells but also B-cells and macrophages, was present within the cerebellar white matter and posterior columns. The second patient had a normal cerebellum but

substantial posterior column degeneration, with a “sparse inflammatory infiltrate” present in the peripheral nervous system.

Tijssen *et al* (Tijssen, Thom et al. 2000) presented their pathologic findings in three patients with cortical myoclonus. Two of these patients had proven CD and the other may have had CD as suggested by low B_{12} levels antemortem. There was cerebellar atrophy and degeneration, and strikingly, Purkinje cell loss and Bergmann gliosis, more marked in the superficial regions. The primary sensory, motor, and premotor cerebral cortices, were normal except for unilateral gliosis of the motor cortex in one case.

2.6 Treatment Potential

2.6.1 Gluten free diet

The established importance of treatment with a gluten-free diet in CD is that patients benefit from normal small bowel absorption. In addition, DH patients experience a resolution of the rash although for reasons that are not entirely clear, the response is much slower. A GFD also reduces the risk of development of lymphoma in coeliac patients (Holmes, Prior et al. 1989). Disappointingly, the potential value of a GFD in treatment or prevention remains unproven in the various neurological deficits already described. The effects of a GFD on these patients ranged from reversal of the deficit, stabilisation of the illness to making little or no difference. It might be that there is a therapeutic window of opportunity in which commencement of a GFD is helpful (Gobbi, Bouquet et al. 1992).

Hadjivassiliou and co-workers (Hadjivassiliou, Grunewald et al. 1998) made the intriguing observation that initiation of a GFD did lead to symptom resolution in some of their gluten ataxia cases. They subsequently reported on 43 patients with idiopathic ataxia of whom 26 adhered to a GFD and showed marked improvement in ataxia after a one-year interval (Hadjivassiliou, Davies-Jones et al. 2003). This study was based on one investigator, unblinded to the patients' dietary compliance, who undertook clinical assessment of these patients. Further randomised controlled trials are required to confirm these findings.

In patients with established epilepsy (Ventura, Bouquet et al. 1991; Bardella, Molteni et al. 1994; Lea, Harbord et al. 1995), there is limited evidence that a GFD prevents or stabilises seizures. Gobbi and co-workers (Gobbi, Bouquet et al. 1992) suggested that a GFD seems to control seizures if started near the onset of epilepsy and early in childhood.

Although isolated peripheral neuropathy is uncommon in CD, there is some suggestion that treatment with a GFD may reverse the neuropathy (Kaplan, Pack et al. 1988). A 12-year old girl was reported with 2 episodes of demyelinating neuropathy with accidental introduction of dietary gluten. These reversed completely following re-introduction of GFD (Polizzi, Finocchiaro et al. 2000).

De Santis *et al* (De Santis, Addolorato et al. 1997) described a patient with previously diagnosed schizophrenia who following investigation for weight loss and diarrhoea was found to have CD (positive EMA and jejunal biopsy). A SPECT scan showed hypoperfusion of the left frontal area. Initiation of a GFD resulted in the disappearance of psychiatric symptoms as well as improvement in the scan appearances.

Numerous studies have shown no significant improvement on a GFD (Lu, Thompson et al. 1986; Ghezzi, Filippi et al. 1997). Moreover, many patients have appeared to develop neurological complications despite adhering to a GFD (Cooke and Smith 1966; Beyenburg, Scheid et al. 1998). Of the 10 cases of CD detected by Luostarinen (Luostarinen, Pirttila et al. 1999), all but one were started on a GFD. In six of the ten patients, there was no change in their neurological symptoms. One patient with neuropathy who later died of lymphoma actually progressed neurologically. His neuropathy was felt to be a paraneoplastic manifestation of the lymphoma. Two patients experienced some relief of their neurological symptoms (myopathy and neuropathy respectively) whilst a patient with epilepsy was apparently cured.

2.6.2 Vitamin replacement

Vitamin deficiency is discussed above. Vitamin replacement may play a role in a select group of patients.

2.6.3 Immunosuppressive treatment

There have been a few reports of immunosuppressive treatment in patients with CD and neurological syndromes. This has been helpful in a case of vasculitis (Rush, Inman et al. 1986) and a case of relapsing-remitting brainstem-cerebellar syndrome (Ghezzi, Filippi et al. 1997). Intravenous steroids and immunoglobulins were not helpful in a case of progressive leukoencephalopathy (Beyenburg, Scheid et al. 1998). Burk and co-workers described four patients with AGA positivity, cerebellar ataxia but no evidence of gluten sensitive enteropathy who were treated with intravenous immunoglobulin (Burk, Melms et al. 2001). All four patients were shown to improve within two to three weeks. However this was an unblinded study.

2.7 Discussion

It is possible that improved case ascertainment has resulted in an apparent increase in the prevalence of CD. This has highlighted the changing pattern of presentation of CD with atypical symptoms becoming more prominent. However, as CD becomes more commonly diagnosed, it is likely that some associated diseases may merely be a result of chance. Alternatively, similar HLA haplotypes may confer an increased likelihood of autoimmune disease as exemplified by the increased incidence of hypothyroidism and Type 1 diabetes mellitus in CD, although this too remains uncertain.

The apparent or possible increase in prevalence of a variety of neurological conditions in CD suggests that patients with neurological disease are a target population who might benefit from screening and treatment. A causative

association between gluten sensitivity and neurological disorder are appealing because GFD may potentially be an inexpensive, relatively straightforward treatment option for a number of incurable conditions. However, our knowledge of causality remains uncertain. At present, the available data on neurological associations of CD is extremely heterogeneous with no universally acceptable scientific explanation for a causative effect. The varied literature on neurological complications of CD is flawed for a number of reasons namely referral bias, lack of blinding, difficulty of monitoring GFD for long periods, high prevalence of gluten sensitivity in the general population and also non-standardised serological testing. Furthermore, neurological assessment by non-neurologist is liable to inaccuracy.

At this stage, it is possible that the majority of neurological syndromes have a chance association with CD. There may be a minority with a definite association, such as patients with certain forms of epilepsy, but further study is required to confirm this and the nature of the association. There are clear mechanisms by which patients with true gluten sensitivity can have neurological illness namely malabsorption and malignancy with neurological sequelae. Deficit of a trace element or vitamin might play an important role and this has considerable therapeutic implications. Thus far, this aspect of treatment has not been studied systematically.

Malignancy has been demonstrated as a cause for neurological disease in patients with coeliac-related lymphoma with central neurological spread

(Shams, Waldman et al. 2002). A rarer but scientifically plausible alternative is a paraneoplastic syndrome associated with coeliac-related malignancy.

The role of altered auto-immunity, particularly in susceptible HLA sub-groups, also merits further investigation. Moreover, with increasing recognition that CD is under-diagnosed, it would be prudent to be vigilant in all clinical settings. The concept of a “coeliac iceberg” needs extending with the realisation that the potential for further research into this fascinating area of medicine is almost unlimited.

Chapter 3 - Coeliac disease and coexistent neurological illness: a retrospective study

3.1 Aim

I aimed to carry out detailed case report analysis of patients with CD and coexistent neurological dysfunction from data gathered nationally using the British Neurological Surveillance Unit (BNSU) and from my personal case series of patients recruited in Derby and Nottingham. This was intended to gain some insight into the spectrum of neurological disorders seen with CD

3.2 Introduction

A variety of neurological disorders have been reported in association with CD as discussed above. Mechanisms proposed include heredity, nutritional deficiency and an immune role for gluten. However the nature of this association remains unclear and indeed controversial. There are myriad studies detailing the various neurological diseases that occur with CD. Many of these are on a case report basis. There has been no detailed United Kingdom–wide study of neurological illnesses occurring with CD. Holmes (Holmes 1997) in a survey of 388 adult patients attending a CD clinic found 102 patients (26%) had developed 132 neurological or psychiatric disturbances.

3.2.1 The British Neurological Surveillance Unit

The BNSU was established in January 1992 and is controlled by the Council of the Association of British Neurologists (ABN) who are responsible for the election of members of the BNSU advisory committee. This scientific advisory committee consists of neurologists and one epidemiologist who review

research proposals in order to ascertain the appropriateness of the use of the BNSU for that condition. The main criteria are that the disease should be rare, predominantly seen by the specialists surveyed and of sufficient scientific or public health interest merit. The system aims to provide a comprehensive surveillance system encompassing the whole of the UK in order to identify cases of rare neurological diseases. As a proportion of patients with neurological diseases are under the care of non-neurologists, other professional groups besides neurologists are included in this surveillance and asked to participate, namely paediatric neurologists, neurophysiologists and neuropsychiatrists. Participants are sent reporting cards on a monthly basis and asked to return these if they have seen the disease under investigation.

3.3 Methods

All patients were recruited either via the BNSU or from my own database of patients reported locally. All senior members of the ABN were sent monthly report cards for notification of any cases of neurological disorders seen with CD. Reporting instructions were sent out from March 2001 to March 2003. I contacted each Consultant who had sent a positive return, i.e. a returned card reporting identification of a patient with co-existent neurological disorder and CD, to the BNSU to obtain details of each patient (name and address). I was then able to contact the patient directly in order to obtain signed consent allowing access to their casenotes and to send him/her a copy of the consent form to keep as well as a patient information leaflet. Where consent was not possible e.g. if the patient had died, attempts were made to obtain anonymised casenotes.

My database consisted of patients with co-existent CD and neurological disorder that I had identified myself or that had been identified by my colleagues within the Departments of Neurology at the Queens Medical Centre, Nottingham and the Derbyshire Royal Infirmary, Derby.

Data collected included neurological diagnosis, age and sex of the patient and length of time from diagnosis of CD to diagnosis of neurological disorder.

3.3.1 Ethical considerations

I had Multi-Centre Regional Ethics Committee (MREC) approval from Ethics Committees of both the Queen's Medical Centre, Nottingham and Southern Derbyshire Health Authority, Derby.

3.4 Results

In 24 months of reporting, 23 positive returns were received from the BNSU. This is comparable in rarity to other case series of rare neurological conditions reported via the BNSU e.g. paraneoplastic neurological syndromes (7 cases per year) and Duane's syndrome (8 cases per year). 13 patients were included in my coeliac neurology database from my personal case series and reporting from local colleagues in Derby and Nottingham between April 2000 and October 2002 (31 months). Three patients were unable to give signed consent although I had anonymised notes of 1 patient. 1 patient declined to participate. 1 patient did not return consent. I did not receive patient contact details/casenotes of 15 patients.

I was therefore able to review 18 sets of casenotes. Neurological disorders included epilepsy [4 (22%); 1 with learning difficulty], stroke [3, (17%)], extra-pyramidal disorder [2 (11%)], migraine [2 (11%); 1 with subtle dysphasia and cognitive impairment], myelopathy [1 (6%)], axonal neuropathy [1 (6%)], paraneoplastic ataxia [1 (6%)], Lambert Eaton myasthenic syndrome [1 (6%)], akathisia [1 (6%)], facial numbness [1 (6%)] and memory disturbance [1 (6%)]. All 4 of the patients with epilepsy had had computed tomography of the brain. None had occipital calcifications. No cases of 'gluten ataxia' were reported.

The male:female ratio was 1:1 with mean age 53 yrs 10 months (Range 35 yrs 7 months to 69 yrs 7 months). Details of date of diagnosis of CD, and hence length of treatment with GFD, were not available in the majority of the patients although CD predated the neurological diagnosis in all patients reviewed. However in the 5 patients in whom it was available, CD predated presentation to a neurologist from 9 months to 30 yrs. These findings are summarised in Table 3.1 below.

Patient	**Age**	**Sex**	**Neurological disorder**	**Time from CD diagnosis to neurological diagnosis**
1	38y 7m	M	Epilepsy + mild learning difficulty	30 years
2	48y 2m	F	Myelopathy	N/A

3	61y 10m	M	Axonal neuropathy	24years
4	48y 3m	F	Migraine	9 months
5	47y 10m	F	Stroke	N/A
6	48y 2m	M	Stroke	10 years
7	48y 7m	F	Facial numbness	N/A
8	69y 1 m	F	Parkinson's disease	N/A
9	64y 4m	M	Lambert-Eaton myasthenic syndrome	N/A
10	56y 11m	M	Epilepsy	N/A
11	39y 6m	F	Migraine	N/A
12	65y 2m	M	Venous sinus thrombosis	N/A
13	69y 7m	F	Akathisia	20 years
14	45y 3m	M	Memory difficulty	N/A
15	61y 9m	F	Extrapyramidal syndrome	N/A
16	35y 7m	F	Epilepsy	N/A
17	65y 1 m	M	Paraneoplastic ataxic syndrome	N/A
18	55y 11m	M	Epilepsy	N/A

Table 3.1 Table summarizing age, sex, neurological deficit and time from coeliac disease diagnosis to neurological diagnosis in a series of patients with coeliac disease and neurological illness

3.5 Discussion

The BNSU is a valuable aid in the study of rare disorders but in less severe conditions, such a method of ascertainment inevitably underestimates prevalence and is prone to selection bias towards patients with associated morbidity. This is a major limitation of this study. Furthermore the system depends very much on the enthusiasm of reporting clinicians. My own local case series on the other hand is likely to be prone to ascertainment bias. However the intention of this study was to gain some insight into the scope of neurological disorders seen with CD rather than true prevalence.

CD and coexistent neurological dysfunction is only rarely reported in a neurological setting. This is the first UK-based surveillance study of CD and coexistent neurological dysfunction. BNSU reporting combined with local experience in Nottingham and Derby suggests that the association of CD and neurological disorders is rare and that patients reported in this surveillance study formed a heterogeneous group with the commonest neurological association being epilepsy and stroke. This may suggest an increased incidence of epilepsy and stroke with CD but is more likely to reflect the fact that these are conditions commonly seen in a neurological setting.

Diagnosis of CD and subsequent GFD predated the neurological diagnosis in all cases reviewed suggesting that the neurological disorders were unrelated to gluten exposure. However I did not have sufficient data regarding time from diagnosis of CD to onset of neurological disease, or indeed level of

compliance with a GFD, which may have been a useful surrogate marker of gluten exposure.

Adherence to GMC guidelines for disclosure of information has made retrospective studies like this increasingly difficult. A major factor in data collection was the recent more stringent guideline issued by the General Medical Council (GMC) which states that the researcher should “seek consent to the disclosure of any information wherever …...practicable”. Where obtaining consent is not practicable the guidelines suggest that data can be anonymised, although even in a case such as this ideally patients should be given the opportunity to object.

In order to proceed with building up this case series, where possible I requested signed consent from each patient allowing us to examine his/her case notes. The cases where seeking consent was deemed not practicable were usually communicated to me as such by the reporting consultant on the grounds of recent death of the patient, severe illness or lacking capacity to give consent. I did not attempt to obtain consent from relatives for two main reasons. Firstly, the GMC guidance states that no one can give or withhold consent on behalf of an adult with mental incapacity. Secondly, I did not wish to cause further distress to the recently bereaved families. In these cases I attempted to obtain anonymised data although this proved to be impractical and largely unsuccessful.

To conclude, I did not feel that this small case series supports a hypothesis of a neurological syndrome as a result of CD. However, further study was required to confirm or refute an association between CD and neurological illness.

Chapter 4 - Prevalence of neurological disorders in patients with gluten sensitivity

4.1 Aims

I aimed to examine case records of a large cohort of CD patients in order to establish prevalence of neurological disorders. I also aimed to carry out more detailed investigation of a subgroup of patients from this cohort said to have epilepsy for more accurate prevalence of epilepsy and to discover whether a specific epilepsy syndrome exists in this group of patients.

4.2 Introduction

There have been various descriptions of neurological disorder complicating CD including peripheral neuropathy (Kaplan, Pack et al. 1988) and cerebellar ataxia (Hadjivassiliou, Grunewald et al. 1998). Malabsorption and gluten neurotoxicity are proposed explanations. Studies looking at prevalence of neurological disorders are flawed by differences in study criteria and variable definitions of ‘neurological disorder’. Most prevalence analyses have been performed in selected patient groups in tertiary referral centres. Assessment of neurological disorder by a non-neurologist may be liable to inaccuracy. Interpretation of studies is complicated because CD is common and often occult as most patients with gluten sensitive enteropathy are asymptomatic. One study that included complications secondary to malabsorption found that neurological symptoms occur in 36% of CD patients (Kinney, Burger et al. 1982). Finelli *et al* (Finelli, McEntee et al. 1980) estimated that 10% of patients with coeliac disease develop neurological complications.

4.2.1 Epilepsy and gluten sensitivity

The available published literature suggests an association between CD and certain forms of epilepsy, An increased prevalence of epilepsy in patients with established CD has been reported (Chapman, Laidlow et al. 1978). Studies from Italian centres have suggested an association between CD and epilepsy with cerebral calcifications (Gobbi, Bouquet et al. 1992) although this has not been found in Irish and Finnish populations (Cronin, Jackson et al. 1998; Luostarinen, Dastidar et al. 2001). Low folate levels, attributable to chronic anticonvulsant therapy or malabsorption, have been implicated. Cerebral calcifications are found in other folate deficient states (e.g. following treatment of childhood leukaemia with Methotrexate).

The majority of studies investigating the association of CD with epilepsy have found an increased prevalence of CD in cohorts of patients with epilepsy (Cronin, Jackson et al. 1998; Pratesi, Gandolfi et al. 2003). However, in children with localisation-related epilepsy, only those with occipital paroxysms seem to have an increased prevalence of occult coeliac disease (Labate, Gambardella et al. 2001). The prevalence of epilepsy in CD patients has been reported twice (Chapman, Laidlow et al. 1978; Hanly, Stassen et al. 1982).

4.3 Methods

In 1978 a register of patients with CD in the catchment area of the Derby Hospitals (Derbyshire Royal Infirmary and Derby City General Hospital) was established. All patients diagnosed before this time and subsequently were added to the register. Strenuous efforts were made to identify all patients with

CD in the area and sources included the hospital diagnostic index, pathology records, the Dermatitis Herpetiformis Clinic, the Immunological Laboratory, the Dietetic Department and membership lists of the Coeliac Society. Since 1978, regular fortnightly meetings with the histopathologists have reviewed all small bowel biopsies. It is thus highly likely that the vast majority of patients in the catchment area of the Derby Hospitals with CD have been identified.

A dedicated clinic for CD was set up to act as a focus for patient care and research and has been run personally by Dr GKT Holmes (GKTH) ever since. By the end of 2002, 801 unselected adult patients (over the age of 15 years) with CD had attended the clinic on at least one if not several occasions.

The diagnosis of CD was confirmed by small bowel biopsy in each patient. Case notes were available for review in all of the patients. Of these 91 cases (32 male; mean age at diagnosis 3 years 4 months, range 4 months to 14 years 6 months) were diagnosed in childhood but were referred by paediatricians to the clinic at the age of 15 years for follow up. Of the 710 remaining cases (226 male; mean age at diagnosis 47 years, range 15 to 88 years) diagnosed during adult life, 36 were diagnosed prior to 1978 and all but seven of these who had died were seen in the clinic. Although full notes were available on these seven cases and none appeared to have had any neurological disorders, they were not seen in the clinic and have not been considered in the analysis. A database of all other medical conditions in these patients was compiled and maintained by GKTH by regular case note review. It was therefore possible to identify the types and frequency of neurological conditions in these patients.

21 patients (2.6%) were identified with a lifetime history of seizures. Their case notes were then reviewed to establish the seizure type, frequency and the results of investigations.

4.4 Results

4.4.1 Prevalence of neurological disorders in patients with proven coeliac disease

Of 801 patients within the CD database, 54 neurological disorders were found in 177 patients (22%). The commonest diagnoses are listed in Table 4.1 below. Some patients had more than 1 disorder. The commonest diagnoses found were stroke, migraine, epilepsy and carpal tunnel syndrome which made up 13.1%, 12.6%, 12.0% and 9.1% of the neurological conditions respectively and 2.9%, 2.7%, 2.6% and 2.0% of the total number of patients respectively. No patients with idiopathic cerebellar ataxia were identified.

Neurological disorder	Frequency	% of total neurological conditions (N=175)	% of total patients (N=801)
Stroke	23	13.1	2.9
Migraine	22	12.6	2.7
Epilepsy	21	12.0	2.6
Carpal tunnel syndrome	16	9.1	2.0
Herpes zoster	6	3.4	0.7

Tension Headache	6	3.4	0.7
Sciatica	5	2.9	0.6
Learning disabled	4	2.3	0.5
Parkinson's	4	2.3	0.5
Meningitis	4	2.3	0.5
Deaf	4	2.3	0.5
Multiple sclerosis	4	2.3	0.5
Dementia	3	1.7	0.4
Peripheral neuropathy	3	1.7	0.4
Squint	3	1.7	0.4
Retinal vein (or branch) occlusion	3	1.7	0.4
Benign essential tremor	2	1.1	0.2
Down's syndrome	2	1.1	0.2
Bell’s palsy	2	1.1	0.2
Cubital tunnel decompression	2	1.1	0.2
Tinnitus	2	1.1	0.2
Meniere's	2	1.1	0.2
Others	32	18.3	4.0

Table 4.1 Prevalence of neurological disorders in 801 patients with coeliac disease

4.4.2 Prevalence of epilepsy in patients with coeliac disease

21 patients had a lifetime history of seizures and nine patients had active epilepsy (six focal epilepsy cases, three generalised) as defined by any seizure in the preceding five years. One patient had childhood onset febrile convulsions. The seizure sub-type in the remaining 20 patients was generalised in 12 cases (childhood absences-2, primary generalised in 3,

cryptogenic in 7), focal in seven cases (1 multiple strokes, 1 cortical heterotopia) and secondary to hypoglycaemic attacks complicating diabetes mellitus in one case. The development of CD preceded the onset of seizures in seven out of 21 cases. The onset of seizures occurred at a mean age of 20 years in this cohort of patients (range birth to 87 years), whereas the onset of CD occurred at a mean of 38 years (range 1 to 85 years). All the patients with active epilepsy were on anticonvulsant medication. In those cases where CD preceded the onset of epilepsy the patients had been on a gluten free diet for between 1 to 15 years. The clinical characteristics of the patients are shown in table 4.2 (active epilepsy in bold).

	SEX	CD ONSET YEARS	EPILEPSY ONSET YEARS	SEIZURE TYPE
1	M	16	2	FOCAL
2	F	64	59	CRYPTOGENIC GENERALISED
3	F	25	3	TYPICAL ABSENCE
4	F	52	23	FOCAL
5	F	1.5	2	FEBRILE CONVULSIONS
6	**F**	**61**	**9**	**FOCAL**
7	F	49	7	CRYPTOGENIC GENERALISED
8	F	48	14	PRIMARY GENERALISED
9	**M**	**85**	**87**	**SECONDARY GENERALISED MULTIPLE STROKES**
10	M	1	9	CRYPTOGENIC GENERALISED
11	**F**	**35**	**45**	**FOCAL**
12	**M**	**55**	**PERINATAL**	**FOCAL**
13	**F**	**53**	**PERINATAL**	**CRYPTOGENIC GENERALISED**
14	**M**	**2**	**16**	**PRIMARY GENERALISED**
15	M	53	29	CRYPTOGENIC GENERALISED
16	F	2	1.5	CRYPTOGENIC GENERALISED
17	**F**	**36**	**14**	**FOCAL**

18	F	26	21	PRIMARY GENERALISED
19	**F**	**76**	**19**	**CRYPTOGENIC GENERALISED**
20	F	28	40	HYPOGLYCAEMIA
21	**F**	**43**	**21**	**FOCAL**

CD-coeliac disease, M-Male, F-Female

Table 4.2 Prevalence of epilepsy obtained from a database of an unselected cohort of 801 patients with coeliac disease (Active epilepsy in bold)

4.5 Discussion

In this large database of 801 CD patients, numerous different neurological diagnoses have been recorded in their hospital case notes. However the robustness of these diagnoses were not confirmed. A prevalence of neurological disorder of 22% is higher than has previously been reported. This may approach a true prevalence because great care has been taken to include as much clinical detail regarding these patients into the database as possible. However, some neurological disorders may have been missed e.g. those seen at other hospitals or in primary care. Therefore no firm conclusions are made about the prevalence of each of these conditions from this data apart from epilepsy which was reviewed in more depth.

Epilepsy does not appear to be significantly more common than in the general population in our 801 CD patients. 1.1% of CD patients had active epilepsy compared with a general prevalence of 0.5%-1.0% (Sander 2003). Assuming a mid-point prevalence of 0.73% (Wright, Pickard et al. 2000) (from a background population of 225000) this gives a 95% confidence interval of the difference between the two proportions of –1.39 to 0.14% (p=0.12 - Miettinen

test). Furthermore, no consistent temporal relationship was found between the development of CD and epilepsy.

It is unlikely that these results arise from selection bias because great efforts were made to identify all patients with diagnosed CD in the area. The patients included in this study represent a prevalence of CD in the local background population of approximately 1 in 625. There remain many undiagnosed patients in this area because screening studies give a prevalence of 1% but there is no reason to believe that the frequency of epilepsy is any different in this group.

Previous studies in CD populations have provided conflicting data on the prevalence of epilepsy. Chapman and co-workers identified 185 CD patients and found that nine subjects had active seizures (5.5%), predominantly of complex partial epilepsy (Chapman, Laidlow et al. 1978). Hanly *et al* found a prevalence of epilepsy in 1% of 177 CD patients (Hanly, Stassen et al. 1982). Both of these studies used structured questionnaires sent to the patients prior to neurological review. It could be argued that the figures in this study are under-represented as I relied on information from clinical interviews rather than standardised questionnaires to establish a seizure history.

How CD might predispose subjects to epilepsy is unclear. Some authors have argued that gluten is neurotoxic (Hadjivassiliou, Boscolo et al. 2002), whereas others have suggested that pyridoxine or folate deficiency might lower the seizure threshold (Lea, Harbord et al. 1995). If gluten is neurotoxic, a gluten

free diet (GFD) could reduce the risk of epilepsy in CD. Ventura and co-workers described two cases of refractory focal occipital epilepsy, cerebral calcifications and CD who showed marked improvement on a GFD and folate supplementation (Ventura, Bouquet et al. 1991).

This study highlights a number of points with regards to the association of CD with neurological illness in general and epilepsy in particular. The strength of the study is that it comprises a large series of unselected CD cases comprising all CD patients diagnosed within the catchment area of the Derby Hospitals in Southern Derbyshire. This is not a tertiary referral centre so patients with CD are more likely to be representative of those in the general population. Any further comparison to the general population is less accurate as there are likely to be numerous other confounding variables e.g. access to diagnosis and treatment, differences in demographics and lifestyle. In addition there may be under-reporting of neurological conditions within the database as this was compiled by non-neurologists and casenotes are unlikely to hold all pertinent medical information. However, this may be less of a factor because there was active scrutiny for additional diagnoses in these patients during the course of their follow-up. Conversely there may be reverse causality as patients attending medical practitioners regularly are more likely to be diagnosed with illness even if disease prevalence is the same. Another weakness of this study is that I did not obtain figures for the prevalence of epilepsy in a suitable control group such as in patients with gastro-intestinal reflux.

Review of general practitioner records might provide an alternative method of investigating the question of association of CD and neurological dysfunction. To my knowledge this approach has not been utilised previously, but the General Practitioner Research Database (GPRD) might facilitate such studies in future. However, I feel that the results are likely to be more accurate than those that could be obtained from analysis of the GPRD, because of uncertainties about the accuracy and consistency of GP records documenting the presence of seizures.

In conclusion, this study confirms that numerous neurological disorders are found in a coeliac population. The mechanisms underlying this association remain unclear. No cases of ataxia, (gluten or otherwise) were identified in this cohort of CD patients. The current study suggests that there is no specific association between CD and epilepsy. Further case-control studies are required to determine whether there is a consistently observed association between gluten sensitive diseases and other neurological illnesses.

Chapter 5 – Dermatitis herpetiformis, coeliac disease and neurological dysfunction

5.1 Aims

This study aimed to thoroughly investigate cohorts of patients with CD and DH to determine the prevalence of neurological complications. I propose that if gluten is indeed neurotoxic that patients with DH having chronic gluten exposure should have an increased frequency of neurological disorders.

5.2 Introduction

Patients with CD are well recognised to be at risk of developing complications that increase mortality and morbidity. Whilst malignant complications have been emphasised in the past, it is only in recent years that researchers have turned their attention to other potential associations such as Type I diabetes mellitus, hypothyroidism and osteoporosis. As has been mentioned, prevalence data from studies looking for neurological complications of gluten sensitivity are flawed for a number of reasons including selection criteria and variable criteria for definition of neurological disorder.

Neurological disorders are said to be found in 10% of patients with CD (Finelli, McEntee et al. 1980). However it is likely that detailed neurological assessment would make this figure higher. Malabsorption, whether overt or occult, is a cardinal feature of untreated CD and may result in nutritional deficiencies that potentially play a role in development of neurological deficits. However vitamin deficiency is not always present (Bhatia, Brown et al. 1995), vitamin replacement is not always helpful (Lu, Thompson et al. 1986) and

often no neurological complications occur even with severe vitamin deficiency. No other definite explanation for an association between CD and neurological dysfunction exists although some authors have proposed a neurotoxic role for gluten (Hadjivassiliou, Grunewald et al. 2003). This hypothesis is an unsatisfactory explanation as some patients develop a neurological disorder despite strict adherence to a GFD.

One study found a low prevalence of neurological disorders in 305 patients with DH (Reunala and Collin 1997). Whilst classical CD is a gluten sensitive enteropathy, DH is a blistering skin disease characterised by IgA deposition in papillary dermis. There is robust immuno-pathological and genetic evidence that DH and CD are closely related conditions. DH has a similar incidence of the HLA haplotypes DR3/DQ2 to CD. Twin and family studies also support this association. GFD may be used to treat DH but patients may take up to 2 years to respond to gluten restriction and therefore patients are routinely offered dapsone. In the absence of enteric symptoms, compliance to GFD may be poor. DH tends to present later than CD and thus exposure to gluten is longer. Therefore, if gluten toxicity results in neurological complications, these should also occur in DH patients and potentially with increased frequency compared to CD patients.

5.3 Methods

A group of 35 patients with DH was identified from Dermatology outpatient clinics at St. Mary's Hospital, London and the Queen's Medical Centre,

Nottingham from November 1999 to April 2000. All the latter group of patients had presented with a rash and the diagnosis of DH had been previously confirmed by skin biopsy showing characteristic IgA deposits in the dermoepidermal junction on direct immunofluorescence. These patients were examined by Dr. AJ Wills, Consultant Neurologist and Dr. B. Turner, Specialist Registrar in Neurology.

A description of the register of patients with CD in the catchment area of the Derby Hospitals is given in the previous chapter. From November 2000 to May 2002, I identified 53 patients from the CD clinic at the Derbyshire Royal Infirmary, Derby (set up to act as a focus for patient care and research and run personally by Dr GKT Holmes since inception of the Derby CD register in 1978). Fifty two of these 53 patients had had confirmatory small bowel biopsy except 1 with severe latex allergy in whom serological testing was convincingly positive (IgA AGA and EMA). These patients were examined by me.

Each patient was questioned about previous and current neurological and enteric symptoms. All patients underwent thorough neurological examination and casenote review. Nerve conduction studies were requested where appropriate. All patients gave informed written consent.

5.3.1 Ethical considerations

I had Multi-Centre Regional Ethics Committee (MREC) approval from Ethics Committees of both the Queen's Medical Centre, Nottingham and Southern Derbyshire Health Authority, Derby.

5.4 Results

5.4.1 Neurological abnormalities in patients with dermatitis herpetiformis

Thirty five patients with confirmed DH were identified with mean age 59 years (range 13-81) and mean disease duration 20 years (range 3-42). Thirty two patients had had intestinal biopsies of which 27 (84%) showed villous atrophy. Only 11 of these 27 had ever reported any gastrointestinal symptoms. Fourteen of the 35 patients were controlled by a GFD alone although compliance may have been poor in a proportion of these patients. Fifteen patients required dapsone as well as adhering to a partial GFD. Six patients chose not to take a GFD and the rash was controlled with dapsone alone. Neurological abnormalities that were identified in the DH cohort were chorea (1), essential tremor (1) and migraine (2). The patient with chorea had been taking long term phenytoin after a single seizure in 1985 at the age of 61. The clinical details are summarized in Table 5.1 below.

Patient No	**Age**	**Disease Duration**	**Sex**	**Dapsone**	**Neurological abnormality**
1	81	4	M	Yes	None
2	55	37	M	Yes	None
3	61	3	F	Yes	**Migraine**
4	66	7	F	Yes	None
5	51	8	M	Yes	None
6	13	4	F	Yes	None

7	63	26	F	No	None
8	45	7	M	Yes	None
9	65	30	F	No	None
10	62	30	F	Yes	None
11	78	27	M	No	None
12	36	21	F	Yes	None
13	37	5	F	Yes	None
14	64	12	M	No	None
15	60	17	M	No	None
16	59	42	M	Yes	None
17	73	4	F	Yes	None
18	64	12	M	No	None
19	52	14	M	Yes	None
20	67	27	M	Yes	None
21	69	22	F	No	None
22	65	29	M	No	None
23	73	10	M	Yes	None
24	74	30	M	Yes	**Essential tremor**
25	56	21	M	No	None
26	48	26	M	Yes	None
27	68	38	M	No	None
28	59	9	F	Yes	None
29	40	16	F	No	None
30	66	20	F	No	None

31	75	25	F	No	**Chorea**
32	59	12	M	Yes	None
33	60	9	M	Yes	None
34	56	38	F	Yes	**Migraine**
35	55	35	F	No	None

Table 5.1 Clinical details of a cohort of 35 patients with dermatitis herpetiformis including any associated neurological disorder

No cases of myoclonus, ataxia, dementia or peripheral neuropathy were detected in this cohort. One further patient with a history of lumbar spine surgery had absent ankle jerks. His nerve conduction studies were normal in the upper limbs and showed only motor abnormalities in the lower limbs suggesting a lumbar radiculopathy secondary to previous surgery (lateral popliteal CMAP 0.2 mV, sural SAP 6μV). Another patient had depressed upper limb reflexes which improved with clinical reinforcement. Nerve conduction studies were normal and this patient was judged normal.

5.4.2 Neurological abnormalities in patients with coeliac disease

53 patients with confirmed CD were identified with a mean age 48 years (range 18-88). Twenty-two patients had newly diagnosed CD (awaiting or newly started on GFD) with mean age 53 (range 21-88). Thirty-one patients had established disease (GFD longer than 1 year) with mean age 45 (range 18-81). Patients already on GFD had received this for a mean of 11 years (range 1-26). Nine neurological abnormalities that were identified in 16 of 53 patients were epilepsy (1), restless legs syndrome (1), carpal tunnel syndrome (1), benign fasciculations (1), multiple sclerosis (1), Sydenham's chorea (1),

cluster headache (1), diabetic neuropathy (2) and migraine (7). Idiopathic neurological dysfunction occurred in 13 of 53 patients overall (25%). In the newly diagnosed group, the prevalence of idiopathic neurological diseases was 6 of 22 (27%) whilst in the established group the prevalence was 7 of 31 (23%). No cases of myoclonus or dementia were detected in this cohort. The clinical details are summarized in Tables 5.2(a) and 5.2(b) below.

Patient Number	**Age**	**Sex**	**Neurological abnormality**
1	53	M	**Epilepsy**
2	42	F	None
3	75	M	None
4	63	M	**Diabetic neuropathy**
5	52	F	None
6	33	F	None
7	45	F	None
8	82	F	None
9	36	F	**Migraine**
10	56	M	**Benign fasciculations**
11	42	F	**Multiple sclerosis**
12	31	M	**Cluster headache**
13	88	F	**Migraine**
14	21	F	None
15	62	M	None

16	61	M	None
17	43	F	None
18	70	F	None
19	49	M	None
20	50	F	None
21	56	F	None
22	53	M	**Diabetic neuropathy**

Table 5.2(a) Clinical details of 22 patients with newly-diagnosed coeliac disease including any associated neurological disorder

Patient No	Age	Disease Duration	Sex	Neurological abnormality
23	40	1	F	None
24	47	10	F	None
25	77	5	F	None
26	18	14	F	None
27	44	15	F	None
28	65	7	F	None
29	54	16	F	**Restless legs syndrome**
30	60	6	M	None
31	48	6	F	None
32	37	5	F	None
33	41	5	F	**Carpal tunnel syndrome**
34	55	17	F	None
35	53	26	F	None
36	27	5	F	None

37	47	18	F	None
38	60	21	F	**Migraine**
39	28	21	F	None
40	38	10	F	**Migraine**
41	30	1	F	None
42	63	11	F	None
43	32	1	F	None
44	70	8	F	None
45	34	13	F	None
46	48	21	F	None
47	42	1	F	None
48	26	25	F	**Sydenham's chorea**
49	26	25	F	**Migraine**
50	45	1	M	None
51	30	7	F	**Migraine**
52	39	15	F	**Migraine**
53	81	7	F	None

Table 5.2(b) Clinical details of 31 patients with established coeliac disease including any associated neurological disorder

Two patients, both with newly-diagnosed CD, had diabetic neuropathy. One (patient 4) was a non-insulin dependent diabetic and asymptomatic but was found to have areflexia and reduced pinprick and vibration sense in a stocking distribution. The other (patient 22) was insulin dependent and had other evidence of end-organ disease namely diabetic retinopathy. Two other

patients had diabetes (patient 1 diet-controlled and patient 44 with longstanding insulin-dependent diabetes) but neither had symptoms or signs of neuropathy.

The patient with epilepsy was stable on phenytoin. It was therefore not felt that brain imaging to exclude cerebral calcifications was justifiable.

Of interest was the patient with Sydenham's Chorea (patient 48). She had presented at the age of 15 in 1990 with short-lived ataxia and chorea following an acute illness with headache and vomiting. At the time she was seen by a Consultant Neurologist who made this diagnosis. She had been established and compliant with a GFD since the age of 3 years. On formal neurological examination there are currently no abnormalities detectable. Her twin sister was also included in this study (patient 49). She had not suffered with the same ataxic illness as her twin.

The patient with benign fasciculations (patient 10) was discovered to have these on examination in the course of this study. He has not shown any neurological deterioration. His voltage-gated potassium channel antibodies were negative.

Another patient was found to have absent ankle jerks bilaterally with reduced vibration sense on the left. There was a history of sciatica. His nerve conduction studies were normal. One further patient had absent upper limb

reflexes but normal nerve conduction studies and this patient was also judged normal.

5.5 Discussion

This retrospective study has shown a low prevalence of idiopathic neurological abnormalities in DH (4/35, 11%). Essential tremor (1/35) and migraine (2/35) are sufficiently common in the general population for this to have been a chance association. The prevalence of essential tremor may be as high as 50/1000 in those older than 60 (Louis, Ottman et al. 1998). Twenty patients in the DH cohort were over 60. The 1 year prevalence of migraine is 25% (Launer, Terwindt et al. 1999). There have been isolated case reports of patients with chorea associated with chronic phenytoin administration. However chorea has also been reported in association with CD (Pereira, Edwards et al. 2004). This patient had been on a strict GFD with no evidence of dietary indiscretion so it is suggested that gluten toxicity did not play an aetiological role.

The prevalence of idiopathic neurological disorder in the CD cohort is 25% (13/53) however, the DH group is not significantly different from the CD group (Fisher's exact test p=0.17). The prevalence of neurological disorder is higher than the DH cohort but is probably skewed by the high prevalence of migraine. However, as mentioned above, migraine is relatively common. There is a conflicting literature with regards to prevalence of migraine in CD with some authors supporting an association (Cicarelli, Della Rocca et al. 2003) whilst others not (Gabrielli, Cremonini et al. 2003). Similarly, restless legs syndrome, carpal tunnel syndrome, multiple sclerosis and cluster

headache are also sufficiently frequent to be chance associations with estimated prevalences of 10.6% (Berger, Luedemann et al. 2004), 9.2% (de Krom, Knipschild et al. 1992), approximately 1 in 500 in Scotland (Rothwell and Charlton 1998) and 1 in 500 (Russell 2004) respectively.

The underlying association between benign fasciculations and CD is not known and has not been previously reported. Whilst it is now recognised that the cramp-fasciculation syndrome has an auto-immune basis (Newsom-Davis, Buckley et al. 2003) this patient was asymptomatic, did not progress and his voltage-gated potassium channel antibody was negative and hence no auto-immune association can be inferred.

The prevalence of epilepsy is discussed in the previous chapter. General prevalence of epilepsy is 0.5 to1% compared to 2% (n=1) in this cohort of 53.

No cases of gluten ataxia or idiopathic neuropathy were seen in either the DH or CD cohorts.

If gluten is neurotoxic, patients with a greater gluten exposure should be at higher risk of neurological complications. The lack of association between DH and neurological sequelae is therefore an important finding as these patients often have no enteric symptoms, and their skin symptoms may be well-controlled on dapsone alone. Furthermore the cohort of newly-diagnosed CD patients was older (mean age 53 compared to patients established on GFD with mean age 45) and therefore overall also had a long exposure to gluten.

There was no significant difference between prevalence of neurological disorders in the newly-diagnosed and established cohorts of CD patients.

This study does not support a role of gluten in causing neuronal damage. Certainly it challenges the notion of a dose-related effect. No evidence of immune-mediated neurological damage other than the patient with likely post-infectious chorea was found. The role of disordered biopterin synthesis or trace element deficiencies such as thiamine, niacin and pyridoxine, has not been analysed in this study.

The numbers of patients in this study was relatively small. However each case was subjected to detailed history-taking and examination by a neurologist and hence there is a degree of accuracy pertaining to each subject's neurological status. The higher prevalence of neurological disorders seen in these patients compared to those described in the previous chapter is likely to be accounted for by this being neurologist-led as opposed to the database/notes review described in the previous chapter which may be flawed by less sensitive identification of neurological disorder by non-neurologists.

An important deficiency is the lack of a gastroenterological control population such as patients with irritable bowel syndrome (IBS).

To conclude, it was felt that the neurological abnormalities found in these patients are likely to be either chance or spurious associations. No cases of gluten ataxia were discovered nor was there evidence of immune-mediated

neurological damage. The hypothesis of a relationship between DH and CD with neurological abnormality remains unproven.

Further prospective studies should be carried out in large populations, ideally as case control studies. The nature of association of migraine and CD remains contentious (Cicarelli, Della Rocca et al. 2003; Gabrielli, Cremonini et al. 2003) and further investigation into putative association is recommended. On the basis of this study, I suggest that the role of pathological mechanisms should be further explored such as the role of malabsorption of biopterin compounds and trace vitamins and the role of altered auto-immunity and in particular susceptible HLA groups.

Chapter 6 - A novel anti-spinal antibody in patients with gluten sensitivity

6.1 Aim

I aimed to investigate the presence of anti-neuronal antibodies in serum of patients with confirmed gluten sensitivity namely patients with established DH and CD.

6.2 Introduction

The association of CD with a variety of neurological disorders has been frequently described but whether a specific neurological association occurs with CD remains unproven. The aetiology of these associations is unclear. Gluten neurotoxicity has been postulated as a mechanism to explain the apparent association of gluten sensitivity with various neurological disorders described prominently with cerebellar ataxia (‘gluten ataxia’) (Hadjivassiliou, Grunewald et al. 1998). It has been suggested that the basis of this is immunological.

Enteropathy in classical CD is presumed to be immunological and T-cell mediated. Immune mechanisms such as deposition of circulating immune complexes in other organs are a likely cause of extra-intestinal manifestations of gluten sensitivity. For example, the blistering skin condition, DH, also a gluten-sensitive disease, is characterised by IgA deposition in papillary dermis. Previous chapters have demonstrated a low prevalence of idiopathic neurological abnormalities in patients with DH and CD with no evidence of immune-mediated neurological damage. The relationship between gluten

exposure and neurological abnormality is questionable, particularly with regards to immune-mediated neurological damage.

6.3 Methods

6.3.1 Patients

Thirty-five patients with biopsy-proven DH were identified from Dermatology outpatient clinics at St. Mary's Hospital, London and Queen's Medical Centre, Nottingham. Fifty-three patients with biopsy-proven CD were identified from a CD outpatient clinic at the Derbyshire Royal Infirmary, Derby. Patients underwent thorough neurological examination and casenote review. Their clinical features are described in the previous chapter. The subjects' consent was obtained according to the declaration of Helsinki and ethical approval was obtained from the Ethics Committees of both institutions. Serum samples were stored at -20˚C until required

6.3.2 Controls

Thirty random anonymous blood donors, 22 non-CD patients with idiopathic neurological disorder (ataxia and peripheral neuropathy) and 30 patients with psoriasis were used as controls.

6.3.3 Antineuronal antibody Western Immunoblotting

The presence of anti-neuronal antibodies was investigated in the serum of the gluten-sensitive group (CD and DH) as well as the control patients. Samples and controls were diluted 1:300. Proteins extracted from guinea pig brain were stored at -70˚C before use. Samples of the homogenate were reduced and

denatured in lithium dodecyl sulphate buffer (Invitrogen) at 65˚C for 15 minutes. A total of 30µg of protein was loaded onto a 4 to 12% Bis-Tris gel (Invitrogen) and electrophoresed. The proteins were blotted onto nitrocellulose (Sartorius, Epsom) and blocked by incubation in a 2% solution of powdered cow's milk for 2 hours. Samples were diluted 1:300, applied to the blot, and incubated overnight at 4˚C. The nitrocellulose was washed with 10 changes of 0.9% saline containing 0.2% milk proteins and Tween. The blot was incubated for 2 hours with rabbit anti-human IgG conjugated with horseradish peroxide diluted at 1:1000 (Dako, Cambridge, UK). After washing, the substrate 4-chloro-1-naphthol (Sigma) was added and the blot was allowed to develop for 15 minutes. This technique is routinely used in the Neuroimmunology Laboratory, Institute of Neurology, Queen Square for identifying antibodies associated with paraneoplastic disorders.

The same method was used to examine binding to spinal cord using human spinal cord extract.

6.4 Results

Fourteen of the 35 DH patients were controlled by a GFD alone although compliance may have been poor in a proportion of these patients. Fifteen patients required dapsone as well as adhering to a partial GFD. Six patients chose not to take a GFD and the rash was controlled with dapsone alone. Neurological abnormalities that were identified in the DH cohort were chorea (1), essential tremor (1) and migraine (2).

53 patients with confirmed CD were identified with mean age 48 years (range 18-88). Twenty-two patients had newly diagnosed CD (awaiting or newly started on GFD) with mean age 53 (range 21-88). Thirty-one patients had established disease (GFD longer than 1 year) with mean age 45 (range 18-81). Patients already on GFD had received this for a mean of 11 years (range 1-26). Nine neurological abnormalities that were identified in 16 of 53 patients were epilepsy (1), restless legs syndrome (1), carpal tunnel syndrome (1), benign fasciculations (1), multiple sclerosis (1), Sydenham's chorea (1), cluster headache (1), diabetic neuropathy (2) and migraine (7).

6.4.1 Laboratory Findings

On testing for binding to guinea pig brain, the only positive finding was in the DH patient with chorea who had equivocally positive Anti-Hu antibodies. An unexpected finding was a novel anti-spinal antibody detectable from peripheral blood in over 50% (18/34) of the subjects with DH. On Western blot, this antibody reacted with a protein of 74 kDa from an extract of human spinal cord. This antibody was not found in CD patients, non-CD patients with idiopathic neurological disorder, psoriasis patients or blood donor controls.

6.5 Discussion

CD is an immune-mediated disorder and as such an immune mechanism for neurological illness in patients with proven gluten sensitivity is an attractive one. Evidence for this is conflicting particularly the role of anti-neuronal antibodies. One criticism of previous studies is a poor understanding of the accepted pathogenic mechanisms of the immune reaction that occurs with CD and DH. In fact these are not completely understood. We know that TTG

seems to be the target self-antigen for endomysial antibodies in CD and catalyses the critical deamidation of gliadin (the toxic fragment of gluten, the protein fraction of wheat) which strengthens its recognition by HLA-restricted gut-derived T cells. A recent study demonstrated that gliadin was directly bound to TTG in duodenal mucosa of coeliacs and controls, and that circulating TTG-autoantibodies were able to recognize and immunoprecipitate the TTG-gliadin complexes (Ciccocioppo, Di Sabatino et al. 2003).

Volta and co-workers (Volta, De Giorgio et al. 2002) analysed a large series of adult CD pts with a variety of neurological disorders in comparison to CD patients without neurological disorder. They found antineuronal antibodies in 8 of a heterogeneous group of 13 patients with neurological disorder ranging from patients with non-specified memory deficit to myotonic dystrophy to multiple sclerosis as well as biopsy-proven CD. However they did not make any comparison to a control neurological population and hence the significance of this finding is questionable. They demonstrated a nuclear and cytoplasmic staining of Purkinje cells as well as a nuclear staining of the granular layer of neurones. They did not detect any difference between prevalence of antineuronal antibodies to the enteric nervous system in patients and controls. They further claimed that these patients improved on GFD when in fact some of these conditions usually have no clear treatment e.g. moyamoya that was said to improve with Aspirin and GFD. Overall this study contributed little other than to say that there was some non-specific immune reaction in the nervous system (central and enteric) of a proportion of a small group of patients with CD and neurological illness. One interesting

finding was that these antibodies disappeared a year following GFD and this does merit further attention.

One study has suggested that the presence of circulating Purkinje cell antibodies in the serum of patients with gluten ataxia provides evidence for an immune pathogenesis. The same research has shown that antigliadin antibodies cross react with Purkinje cells, suggesting the sharing of common epitopes between gliadin proteins and Purkinje cells (Hadjivassiliou, Boscolo et al. 2002).

DH, the classical extra-intestinal manifestation of gluten sensitivity, is not fully understood but what evidence exists suggests precipitates of IgA in the papillary dermis containing epidermal transglutaminase. Epidermal and tissue transglutaminases in DH and CD respectively are highly homologous. Cross-reactivity of the two antibodies may explain why patients with either DH or CD will have antibodies to both these enzymes but in DH the skin manifestation occurs because epidermal transglutaminase antibodies produced are of high avidity and affinity (Karpati 2004). This is an intriguing finding and further supportive evidence would be the demonstration of TTG antibodies in patients with neurological gluten sensitivity, specifically a similarly homologous antibody, a neuronal transglutaminase antibody.

A novel anti-spinal antibody was seen only in a high proportion of patients with DH. It has been shown that neurological disorders have a low prevalence in this cohort. No cases of idiopathic cerebellar ataxia were detected. The first patients with CD and neurological abnormality described in detail had

predominantly sensory ataxia (Cooke and Smith 1966). The unexpected discovery of a novel spinal antibody in DH patients may suggest a potential mechanism for the previously reported increased association of sensory ataxia in patients with established gluten sensitivity. However none of the study patients had clinical evidence of dorsal column dysfunction, which may suggest that an additional co-factor (possibly a trace vitamin) is required for the development of symptoms. Malabsorption of some vitamins has been investigated but the role of a number of trace elements has not been elucidated.

DH patients have greater exposure to gluten than CD patients because they often have no gastrointestinal symptoms and their dermatological symptoms are more easily treated with dapsone. The absence of this spinal cord antibody in patients with CD may reflect this. However, the presence of this spinal antibody in DH patients appears to be irrespective of their gluten consumption status. Moreover 22/53 of our CD patients had recent or ongoing gluten consumption (i.e. newly-diagnosed CD patients). It would have followed that this spinal antibody would be found in the newly-diagnosed CD patients also. Therefore this antibody does not appear to be related to gluten exposure.

Further characterisation of this novel spinal antibody is required. Firstly this finding needs to be validated. The next step is immunohistochemistry. Preliminary data on a proportion of these patients suggests binding to dorsal column neurones although this finding needs to be confirmed across all the

samples (Personal communication Prof F Scaravilli). Finally the relevant antigen will need to be identified. This may be a chance finding in a cohort of patients with autoimmune disease and subsequently a link to gluten immunoreactivity would need to be demonstrated.

In conclusion, whilst it is possible that this spinal cord antibody may be an explanation for sensory ataxia in patients with gluten sensitivity, this finding requires replication and further clarification particularly in terms of an additional co-factor for the development of symptoms. Alternatively these patients may simply be exhibiting some non-specific immune reaction in the nervous system as an epiphenomenon.

Chapter 7 – Prevalence of gluten sensitivity in multiple sclerosis

7.1 Aim

I aimed to investigate seropositivity for AGA, TTG and EMA in an unselected group of multiple sclerosis (MS) patients in order to investigate prevalence of occult gluten sensitivity.

7.2 Introduction

Improved screening for IgA antibodies associated with CD i.e. ARA, AGA, EMA and TTG, has improved the detection of CD in recent years. However, IgG class antibodies, have poor disease specificity (Lagerqvist, Ivarsson et al. 2001). AGA is an anti-wheat protein antibody, which, like other food antibodies (e.g. anti-ovalbumin), especially of IgG class, is not disease-specific.

7.2.1 A putative association between ataxia and gluten sensitivity

Associations between AGA positivity (as distinct from CD) and cerebellar ataxia have been reported (Hadjivassiliou, Grunewald et al. 1998), with speculation that the ataxia is gluten induced. Some investigators have further suggested that a GFD is likely to be of benefit in idiopathic ataxic syndromes. However AGA positivity is also seen in a number of ataxias known to have a non-gluten sensitive pathogenesis including autosomal dominant cerebellar ataxia and multiple system atrophy (Abele, Burk et al. 2002).

7.2.2 Multiple sclerosis and occult gluten sensitivity

Previous researchers have investigated the role of a GFD in the treatment of MS and found no benefits (Jones, Pallis et al. 1979). The relapsing-remitting

natural history of MS can make interpretations very difficult. Two patients seen in the Neurology outpatient clinic (1 at the Derbyshire Royal Infirmary, Derby and the other at the Queen's Medical Centre, Nottingham) with MS-like disease were identified who were incidentally discovered to have occult CD**.**

7.2.2.1 Patient 1

A 24 year-old woman presented with diplopia, left retro-orbital pain and right arm and leg weakness. Four months earlier she had developed lumbar and buttock pain and paraesthesia affecting the whole left lower limb with urinary urgency and incontinence, which resolved without treatment. On examination she had left sixth and seventh nerve palsies, ataxia and a mild right hemiplegia. A clinical diagnosis of MS was made. MRI of the lumbar spine was normal. MRI scan of the brain and spinal cord showed an ill-defined high signal area in the left side of the pons (Figure 7.1). CSF protein, glucose and cell count were normal. There were no oligoclonal bands in the CSF but IgG levels were raised suggestive of intrathecal synthesis. Subsequently, routine autoantibody screening revealed positive ARA. She was therefore screened for GSE and was found to have strongly positive IgA EMA, IgA anti-TTG and IgG AGA in the serum. IgA AGA was negative. Interestingly her CSF was also positive for IgA anti-TTG. A subsequent small bowel biopsy was characteristic of GSE and she was commenced on a GFD.

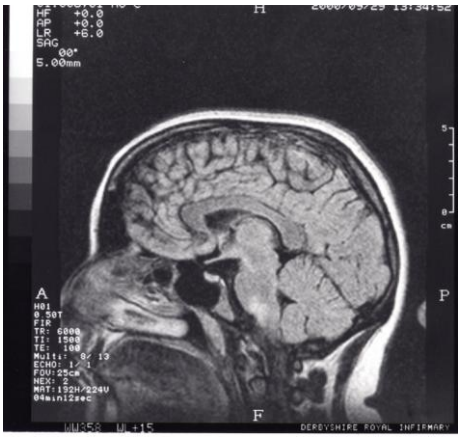


Figure 7.1 T2 weighted MRI of the brain showing high signal area in left side of pons in Patient 1.

7.2.2.2 Patient 2

A 26 year-old woman was admitted with gradual onset of slurred speech, clumsiness and weakness of the right side. Examination revealed cerebellar dysarthria, right-sided weakness, and ataxia. MRI scan of the brain showed a high signal lesion in the right parietal lobe adjacent to the posterior horn of the right lateral ventricle. Antibody screening revealed strongly positive IgA ARA and IgA EMA. IgA and IgG AGA were negative. A duodenal biopsy confirmed GSE and she commenced GFD with correction of the previous borderline low vitamin B12. She was subsequently found to have negative oligoclonal bands in her CSF. Three months later she developed further mild right-sided weakness and subjectively altered sensation of the right-sided limbs and trunk. MRI of the cervical spine showed two high signal lesions in the cervical cord at the level of C2/3 and C4/5 on T2 weighting (Figure 7.2).

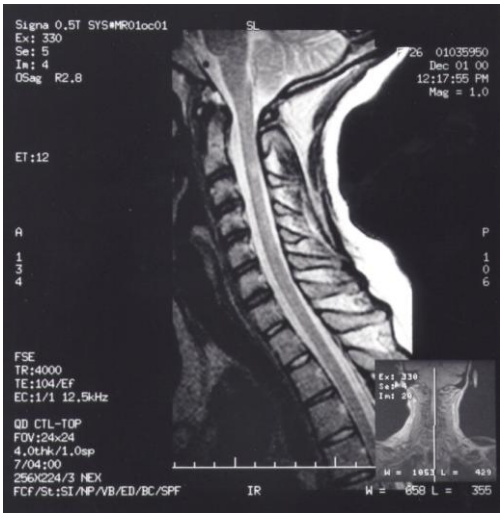


Figure 7.2 T2 weighted MRI of the cervical cord of Patient 2 showing two high signal lesions at C2/3 and C4/5

Both patients were acutely treated with 1g of IV Methylprednisolone daily for three days and had experienced no further neurological episodes at the time of writing.

7.3 Methods

Patients were recruited from general neurology and MS clinics at the Queen's Medical Centre, Nottingham and the Derbyshire Royal Infirmary, Derby. The subjects' consent was obtained according to the declaration of Helsinki and ethical approval was obtained from the Ethics Committees of both institutions.

Forty-nine patients with MS (33 female) were recruited. Thirty-eight had relapsing-remitting disease, 10 had secondary progressive disease and 1 had primary progressive disease. In all cases a Consultant Neurologist with an interest in demyelinating disease made the diagnosis of MS. None of the patients had specific symptoms suggestive of GSE, any suggestive family

history or features suggestive of malabsorption. Thirty random anonymous blood donors (15 female) were used as serological controls.

7.3.1 Immunological testing

AGA (IgG and IgA) and IgA anti-TTG were detected by enzyme linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Orgentec Diagnostika GmbH, Mainz, Germany). These assays were carried out courtesy of Dr RJ Lock, Clinical Scientist and Dr. DJ Unsworth, Consultant Immunologist at the Department of Immunology and Immunogenetics, Southmead Hospital, Bristol.

7.3.2 Statistical testing

Data was analysed by Fisher's Exact Test to compare patients and controls.

7.4 Results

IgG and IgA AGA were found in 6/49 (12%) and 3/49 (6%) patients, respectively, similar to controls (13% and 7%)(p=1.00 and 1.00 respectively). IgA anti-TTG was found in 3/49 patients, again similar to controls (0/30; p=0.466). Of these, two were weakly positive and subsequently found negative for IgA EMA. Serum from one patient was strongly positive for IgG and IgA AGA and strongly positive for IgA anti-TTG and EMA, consistent with CD. This patient had no gastrointestinal symptoms and declined small bowel biopsy. One control subject was positive for IgG and IgA AGA, but negative for anti-TTG and EMA.

	MS patients (n=49)	Controls (n=30)	P value (Fisher's Exact

			Test)
IgA AGA	3 (6%)	2 (7%)	1.00
IgG AGA	6 (12%)	4 (13%)	1.00
IgG anti TTG	3 (6%)	0 (0%)	0.466
IgA EMA	0 (0%)	0 (0%)	

Table 7.1 Comparison of the incidence of AGA, TTG and EMA antibodies in serum of multiple sclerosis (MS) patients and controls.

7.5 Discussion

Only one MS patient (2%) in this study had strongly positive IgA anti-TTG and EMA and it is likely that this patient has occult GSE. This patient had no enteric symptoms and declined small bowel biopsy. CD is common in the general population with a prevalence rate estimated at 1% in the UK. Finding one patient among a cohort of 49 with occult CD is therefore likely to be a chance association.

There is strong historical evidence that occult gluten sensitivity does not play a role in the development of MS. In the 1970s, there was great interest in the possibility of treating multiple sclerosis with a gluten free diet. In 1974, Matheson reported success with a GFD in MS (Matheson 1974) although little was known about the intestinal morphology or antibody status of the patients investigated. Lange and Shiner (Lange and Shiner 1976) showed that two of 12 MS patients had abnormal bowel mucosa. However, in 1979, Jones et al (Jones, Pallis et al. 1979) studied jejunal biopsies in 14 patients with MS, finding no serological or morphological evidence of CD to support the use of a

GFD in MS. Other researchers supported this finding (Bateson, Hopwood et al. 1979; Hewson 1984; Hunter, Rees et al. 1984). Mainstream neurologists have largely abandoned the use of a GFD in MS. Despite this, some MS literature and Internet sites continue to cite the usefulness of GFD and anecdotally a few of our patients claimed to have heard of the diet for disease modification.

In the two patients described with a MS-like illness, occult CD was only suspected following serological clues. Interestingly one patient had positive TTG antibodies in the CSF (not previously reported) raising the question of a pathogenic role for TTG in the development of CNS inflammation.

There have been varying reports of immune-mediated damage to the CNS demonstrable in the CSF of patients with gluten sensitivity. Ghezzi et al (Ghezzi, Filippi et al. 1997) described a patient with CD who developed a relapsing-remitting brainstem and cerebellar syndrome following an intestinal relapse. Serial MRI scanning revealed multiple enhancing lesions. CSF examination, initially normal, subsequently showed the presence of oligoclonal IgG bands and an increased CSF/serum albumin ratio indicating damage to the blood-brain barrier. The authors suggested an inflammatory process as an explanation for neurological complications of patients with CD. It could be argued that this patient had coincidental CD and MS. However, it is noteworthy that this patient's bowel disorder failed to respond to a GFD, which raises diagnostic uncertainties.

Chinnery and co-workers (Chinnery, Reading et al. 1997) reported a patient with CD and Ramsay Hunt syndrome (progressive myoclonic ataxia and epilepsy) with IgG and IgA AGA found in the CSF. They suggested that this finding in the absence of evidence of a generalised breakdown in the blood-brain barrier supported an autoimmune aetiology and local production of AGA within the CNS. A subsequent study looking for positive gliadin antibodies in serum of patients with sporadic ataxia found no inflammatory changes in the CSF of these patients that would account for the neurological symptoms (Burk, Bosch et al. 2001). However, an earlier description by Bernier and colleagues of two women with CD and sensory ataxic polyneuropathy showed a meningeal inflammatory reaction in the CSF of one of the cases (Bernier, Buge et al. 1976). Nerve and muscle biopsies showed inflammation with selective micro-vasculitis in both patients possibly suggesting a mechanism of circulating immune complexes related to CD.

Both patients described above admittedly had a slightly atypical phenotype for MS. It seems more likely that the presence of anti-TTG in the CSF of this patient was secondary to leakage through a damaged blood brain barrier rather than implying a pathogenic role for TTG in the development of CNS inflammation. However I cannot exclude the possibility that these two patients have developed an inflammatory disease of the CNS associated (directly or indirectly) with gluten sensitivity.

In 1996, it was reported that 57% of patients with cryptogenic neurological disorders were AGA seropositive (IgG and or IgA) compared to 5% of patients

with neurological diseases of known aetiology, and 12% of normal controls (Hadjivassiliou, Gibson et al. 1996). A new syndrome “gluten ataxia” was proposed, implying a gluten-mediated immunopathology largely on the basis of AGA seropositivity.

The use of AGA antibodies, particularly IgG AGA, as a screening test for gluten sensitivity remains controversial. Specificity for coeliac disease (gluten enteropathy) is low in comparison with IgA ARA, AEA and anti-TTG. AGA antibodies have been found in a number of conditions such as IgA nephropathy (Rostoker, Laurent et al. 1988). In addition the predictive value of a positive result when applied to the likelihood of developing histopathological features of CD in the gut has been shown to be 28% for IgA AGA in one study (Valdimarsson, Franzen et al. 1996) and 0 % for IgG AGA in another(Collin, Helin et al. 1993). The other difficulty when applying screening to specific populations is that occult gluten sensitivity (as defined by AEA positivity) is common and has been shown to have a prevalence rate of as high as 1:82 individuals in certain populations (Cook, Burt et al. 2000). Recent published reports have suggested that there is a high prevalence of AGA positivity in patients with various genetic (Bushara, Goebel et al. 2001) and sporadic ataxias (Abele, Burk et al. 2002) of known aetiology. This may indicate that AGA positivity is an epiphenomenon, unrelated to neuronal damage.

16% of the MS patients and 17% of the blood donor controls had AGA, mainly of IgG isotype, reflecting the long established poor disease specificity for IgG AGA. IgG AGA seen in other neurological populations should therefore be

interpreted with caution. This is an important observation as some investigators have suggested that a GFD is likely to be of benefit in idiopathic ataxic syndromes. Convincing proof however has not been presented, and this data suggests that this is a false premise.

Chapter 8 - Prevalence of gluten sensitivity in neurological populations

8.1 Aim

I aimed to study the association between gluten sensitivity, HLA status and neurological dysfunction in neurological patients with ataxia, and neuropathy by using assays of anti-TTG, EMA, AGA as well as other food antibodies. MS patients were used as neurological controls.

8.2 Introduction

As shown in the previous chapter, in MS patients there is a high rate of AGA positivity and this is a non-specific finding. This suggests that there needs to be a more cautious interpretation of AGA positivity in the context of neurological patients where the role of gluten in aetiology remains uncertain and in whom a GFD is thus unlikely to be a helpful intervention.

Associations between AGA positivity, particularly in combination with a HLA DQ2 haplotype, and cerebellar ataxia have been reported. Hadjivassiliou and co-workers have proposed that a large proportion of patients with cryptogenic ataxia has occult gluten sensitivity (Hadjivassiliou, Grunewald et al. 2003). They have also suggested that the majority carried the HLA haplotype DQw2, coining the term “gluten ataxia”. This is a potentially important observation as it suggests that simple dietary manipulation (gluten restriction) provides a potential treatment for a condition hitherto thought of as inexorably progressive. Some studies have supported these initial findings. However, this hypothesis has been challenged because AGA positivity is also seen in a

number of ataxias with a known cause including autosomal dominant cerebellar ataxia, multiple system atrophy and alcohol-related cerebellar damage (Abele, Burk et al. 2002). A recent study showed a prevalence of AGA positivity in 27% and 37% of patients with sporadic and autosomal dominant ataxias (ADCA) respectively (Bushara, Goebel et al. 2001). This group of researchers has also demonstrated a high AGA positivity in patients with Huntington's disease (Bushara, Nance et al. 2004).

One possible interpretation is that AGA positivity might be an epiphenomenon associated with cerebellar damage. All these studies have been heavily reliant on AGA positivity as a screening tool and have inferred that the presence of these antibodies is synonymous with clinical gluten sensitivity. This is contested, particularly as in classical gluten sensitive enteropathy it is well recognised that anti-wheat protein antibodies and other simple food antibodies such as anti-ovalbumin and anti-casein are not pathognomonic. In contrast, in GSE, IgA autoantibodies including ARA, EMA and anti-TTG are highly specific and sensitive (Unsworth 1996; Lock, Pitcher et al. 1999).

8.3 Methods

8.3.1 Patients

Over a two-year period, patients with idiopathic ataxia (27) and peripheral neuropathy (32) attending specialist clinics at the Queen's Medical Centre, Nottingham and the Derbyshire Royal Infirmary, Derby were prospectively identified. In these clinics 5400 new neurology referrals are seen annually, from a background population of 2 million people.

In the ataxia group demyelination, alcohol toxicity, prolonged use of anticonvulsant medication and viral or paraneoplastic causes were excluded. None of the patients had evidence of multiple system atrophy (MSA) type C. All the patients had MRI of the brain and all were screened for Friedreich's ataxia and SCA 1, 2, 3, 6 and 7. However, during the course of investigation seven patients were discovered to have either a strong family history of ataxia (3 cases) or the presence of a known genetic defect (Friedreich's ataxia - 2 cases, SCA 3 - 1 case, SCA 6 - 1 case). In the subsequent analysis, these were utilised as an internal disease control group (Group 1), leaving 20 remaining cases with idiopathic ataxia (Group 2).

In the peripheral neuropathy group (Group 3) diabetes, alcohol excess, drug toxicity, paraproteinaemia, systemic vasculitis, vitamin/nutritional deficiencies and other general medical disorders (connective tissue disorders, diabetes etc) were excluded by appropriate investigation. In all cases the diagnosis was confirmed electrophysiologically. Twenty-four were axonal and eight demyelinating in nature.

All patients gave informed consent and the study was approved by the local Ethics Committees.

8.3.2 Immunological and genetic testing

These assays were carried out courtesy of Dr RJ Lock, Clinical Scientist and Dr. DJ Unsworth, Consultant Immunologist at the Department of Immunology and Immunogenetics, Southmead Hospital, Bristol.

8.3.2.1 Antibody Assays

AGA (IgG and IgA) and IgA TTG were detected by enzyme linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Orgentec Diagnostika GmbH, Mainz, Germany). For other food antigen ELISAs, 100μl volumes of βlactoglobulin or ovalbumin (Sigma, Poole UK) at 20μg/ml in carbonate/bicarbonate buffer were used to coat the plates overnight at 4^0C. All further incubations used 100μl volumes, at room temperature, for one hour. All wash steps used 0·05% Tween/PBS repeated three times. After washing, patient's serum diluted to 1/50 in 0·05% Tween/PBS was applied to duplicate wells. After a further wash, 100 μl of either alkaline phosphatase conjugated goat anti-human IgA (Dako Ltd., Ely, UK) diluted 1 in 500 or alkaline phosphatase conjugated goat anti-human IgG (Dako) diluted 1 in 500 in 0·05% Tween/PBS was added to each well. Sigma 104 substrate was used according to the manufacturer's instructions, and the optical density (OD) read at 405nm.

Cut offs for in house ELISA assays, expressed as optical density were determined by the technique of probability plotting (Lock and Unsworth 2003).

8.3.2.2 Class II antigens

DNA was extracted using the Whatman Bioscience Genomic DNA Purification System according to the manufacturer's instructions. HLA-DR Polymerase Chain Reaction using sequence specific primers (PCR-SSP) was adapted from Olerup and Zetterquist (Olerup and Zetterquist 1992). HLA-DQ PCR-SSP was adapted from Olerup *et al* (Olerup, Aldener et al. 1993). PCR-SSP samples were subjected sequentially to 5 cycles of PCR (96°C for 25s, 70°C for 45s,

72°C for 45s), 22 cycles (96°C for 25s, 65°C for 50s, 72°C for 45s) and 4 cycles (96°C for 25s, 55°C for 60s, 72°C for 120s). PCR-SSP products were electrophoresed through a 1.5% agarose gel at 300v for 20 min.

8.3.3 Statistics

Data was analysed using the Binomial test (to assess differences between the test population and reference data), ANOVA or Fisher's Exact Test, as appropriate.

8.4 Results

Table 8.1 shows the median age, age range, male to female ratio and antibody/HLA status for the three groups. Groups 2 and 3 were not significantly different in mean age (p=0.11) or gender distribution (p=0.31). Group 1, found incidentally during the study, is very small and statistical analysis was considered inappropriate. The data do illustrate however that food antibodies of all types are also found in hereditary ataxia. High prevalence of IgG antibodies particularly was noted, ranging from 15-30%. No IgA TTG was detected in any of the groups.

	Hereditary Ataxia (Group 1)	**Idiopathic Ataxia (Group 2)**	**Peripheral Neuropathy (Group3)**
Number	7	20	32
Male:Female	4:3	15:5	20:12

Median Age (range) (years)	51 (36-70)	62 (34-80)	70 (32-86)
Any AGA (IgG or IgA)	3 (43%)	8 (40%) [0·132]	11 (34%) [0·192]
Any AGA and HLA DQ2 positive (#)	2/4 (50%)	4/8 (50%)	5/11 (45%)
Any anti-lactoglobulin (IgG or IgA)	2 (29%)	6 (30%) [0·156]	8 (25%) [0·224]
Any anti-lactoglobulin and HLA DQ2 positive (#)	1/1	2/6 (33%)	2/6 (33%)
Any anti-ovalbumin (IgG or IgA)	3/7 (43%%)	5 (25%) [1·000]	10 (31%)
Any anti-ovalbumin and HLA DQ2 positive (#)	2/3 (67%)	2/5 (40%)	3/9 (33%)
IgA anti-TTG	0 [1·000]	0 [1·000]	0 [1·000]
HLA DQ2 (*) (#)	4/6 (67%) [0·880]	9/19 (47%) [0·894]	11/28 (39%) [0·432]
HLA DQ8 (**) (#)	2/6 (33%) [0.606]	4/19 (21%) [0.937]	5/28 (18%) [0.815]

Table 8.1 Clinical details and antibody/HLA status for the three groups (Hereditary ataxia, idiopathic ataxia and peripheral neuropathy).
P values (in square brackets) calculated by comparison with peripheral neuropathy control population except (*) and (**) where P values calculated against an English population mean of 48·5 and 18.3.
N.B. DNA samples were not available for all cases (#)

8.5 Discussion

There was a striking absence of serological evidence for occult CD in cases with idiopathic ataxia or neuropathy. No cases were positive for IgA anti-TTG. This is very different indeed to the initial reports of up to 25% by some other groups (Hadjivassiliou, Gibson et al. 1996; Pellecchia, Scala et al. 1999; Luostarinen, Collin et al. 2001), although a lower prevalence of biopsy-proven CD has been reported by one group (Burk, Bosch et al. 2001). By contrast food antibodies, (wheat gliadin, hens' egg albumin, cows' milk lactoglobulin), particularly of IgG type were common in patients with idiopathic neuropathy or ataxia. These data support and extend the findings of Abele *et al* showing a significant increase in AGA in ataxia (Abele, Burk et al. 2002) This might simply reflect an age-related phenomenon or it may alternatively imply a general heightened responsiveness of the gut immune system in these individuals or some problem with gut permeability or inflammation.

Again in contrast to the findings of others there was no over-representation of HLA DQ2 or DQ8, in these cases (Hadjivassiliou, Grunewald et al. 1998; Burk, Bosch et al. 2001). In other defined gluten sensitive disorders (CD, DH) HLA DQ2 is found in >95% of cases (Zubillaga, Vidales et al. 2002). Furthermore, in this study, patients with the HLA DQ2 haplotype in this study were no more likely to have AGA than those without (p=0·606).

Of note, very few patients were found who meet the inclusion requirements of Hadjivassiliou *et al*. for "gluten ataxia". Whereas they describe a group of 28 patients with idiopathic ataxia in 4 years who had AGA (Hadjivassiliou,

Grunewald et al. 1998), 23 of whom were also HLA DQ2 positive, this study only identified 4 patients in 2 years who meet these criteria, i.e. only one-third of the incidence assuming that the Neuroscience Centres in Nottingham and Sheffield serve a similar sized population. Ascertainment bias cannot be ruled out as a contributing factor to the differences in the two sets of data.

These results raise a number of interesting issues with regard to the nosological status of “gluten ataxia”. I disagree with the notion that AGA seropositivity *per se* is synonymous with gluten sensitivity whether it be in neurological or other patient populations. 40% of the patients screened in this study have AGA positivity, within which must be contained the 10-15% of healthy individuals who must also have AGA. In the classical or prototypic gluten sensitive diseases (CD and dermatitis herpetiformis) these antibodies lack specificity and alternative antibody disease markers (IgA anti-tissue transglutaminase) are utilised (Wills and Unsworth 2002). An analogous situation arises with IgE class antibodies that may be detected in person without any allergic symptoms.

Whether AGA positivity mirrors, via immune mediated mechanisms, gluten induced neurological damage is debated. I contend that AGA in hereditary ataxias must be an epiphenomenon. The most appealing aspect of the “gluten ataxia” hypothesis is that it offers the prospect of an exclusion diet as a realistic therapeutic possibility. This non-blind study needs confirmation from studies in other centres. This data so far do not support the view that idiopathic ataxia is a gluten-related disorder and by extension nor does it

support the introduction of GFD. It is not known whether dietary restriction of milk and egg products might have a beneficial effect on similar groups.

Chapter 9 – Discussion and conclusions

9.1 Summary of Findings

The overall objective of my research was to investigate the hypothesis of the existence of a gluten sensitive neurological disease. This thesis aimed to look at the association of gluten sensitivity and neurological illness from a number of viewpoints:

- The features and prevalence of neurological disorders associated with gluten sensitive enteropathy,
- The prevalence of gluten sensitivity in patients with neurological disorders,
- Possible mechanisms to account for the apparent association between neurological disorders and gluten sensitivity.

Chapter 1 summarised CD, its epidemiology, pathogenesis, complications, associations and treatment. This contextualised CD as a gluten-sensitive enteropathy of multi-factorial aetiology. Exposure to gluten and a genetic predisposition are the two pre-requisites for development of the disease. DH, manifesting as a dermatological condition, was described as a useful model of another gluten sensitive syndrome.

Chapter 2 reviewed the literature on neurological conditions proposed to have an association with gluten sensitivity including possible aetiology, pathological findings and potential treatments.

The first study, described in Chapter 3, investigated patients utilising the BNSU as well as from personal case series. This revealed that CD with coexistent neurological dysfunction is rarely reported in a neurological setting. The 18 cases reviewed were heterogeneous and included epilepsy,

myelopathy, axonal neuropathy and migraine. No occipital calcifications were reported in patients with epilepsy. No cases of gluten ataxia were reported.

The second study, described in Chapter 4, investigated the prevalence of neurological disorders in an unselected group of patients with CD with particular emphasis on epilepsy. A combination of patient interviews and retrospective casenote review found 54 neurological disorders in 177 patients in a cohort of 801 CD patients. These included stroke (2.9%), migraine (2.7%), epilepsy (2.6%) and carpal tunnel syndrome (2.0%). Of 21 patients with a history of epileptic seizures, only nine (1.1%) had active epilepsy. No specific epileptic syndrome was identified and a causal relationship between CD and active epilepsy seems unlikely on the basis of these observations.

In the third study contained in Chapter 5, careful neurologist-led assessment of two cohorts of patients with DH and CD found a prevalence of idiopathic neurological abnormalities of 11% and CD 25% respectively. DH and CD share common immunopathological and genetic mechanisms. However, DH patients have potentially greater gluten exposure than CD patients as they tend to present later and often continue to consume gluten in the absence of enteric symptoms. Neurological abnormalities found in these 2 cohorts were heterogeneous and felt to be chance associations. No cases of gluten ataxia were seen nor was there any other evidence of gluten-induced immune-mediated neurological damage.

Whilst enteropathy in classical CD is presumed to be immune-mediated, gluten neurotoxicity via immune mechanisms remains unproven. The fourth study, described in Chapter 6, thus investigated the DH and CD cohorts for the presence of anti-neuronal antibodies using Western blotting. A novel

antibody was identified from peripheral blood in over 50% of the subjects with DH. This reacted with a protein of 74 kDa from an extract of human spinal cord. This antibody possibly suggests a potential mechanism for sensory ataxia, which has been previously described in patients with established gluten sensitivity. However none of the patients had clinical evidence of dorsal column dysfunction, which may indicate that an additional co-factor (possibly a trace vitamin) is required for the development of symptoms. The absence of this spinal cord antibody in patients with CD may reflect greater gluten exposure in DH patients who often continue to consume gluten as their dermatological symptoms may be controlled by dapsone alone.

In the fifth study, described in Chapter 7, screening of 49 unselected MS cases for serological evidence of CD revealed IgA anti-endomysial antibody found in one case (2%). IgG AGA was found in 12% of patients and 13% of blood donors. This demonstrated the poor specificity of AGA (especially of IgG isotype) in a cohort of neurological patients the majority of whom did not have gluten sensitive disease.

In the final study, presented in Chapter 8, AGA was compared with other food antibodies, and with the highly disease specific (IgA) anti-TTG in patients with idiopathic ataxia, and neuropathy. All food antibodies (wheat gliadin, hens' egg ovalbumin, and cows' milk lactoglobulin), particularly of IgG type, were found to be common in patients with idiopathic ataxia and neuropathy. None of the ataxia or neuropathy cases were positive for IgA anti-TTG making occult coeliac disease unlikely. HLA DQw2 was found distributed equally across all subject groups. DQw2 expressing, AGA positive cases were very rare in these clinics. This suggests that CD per se is not commonly associated

with either idiopathic ataxia or idiopathic neuropathy. I was unable to confirm that HLA DQw2 is commoner in cases of idiopathic ataxia or peripheral neuropathy. All food antibodies were more commonly found in these idiopathic cases, raising the question of abnormal gut permeability or gut inflammation or a non-specific age-related effect.

9.2 Limitations

Patient numbers were relatively small and thus the current study may not have been adequately powered to show the effects. No analysis was made of variables such as sex, co-morbidity, drugs, smoking etc. This work was retrospective and the prospective effect of treatment i.e. GFD was not assessed. Case series were liable both to under-reporting and over-reporting of neurological disorder.

9.3 Merits

The strength of this work was that this was neurologist-led with close collaboration with colleagues in numerous specialities namely gastroenterology, immunology, dermatology, neuroimmunology and epidemiology. It thus aims to present a holistic and scientifically sound impression of the nature of the association between gluten sensitivity and neurological disease.

9.4 Recommendations

Firstly, I would recommend more carefully-considered, stringent antibody testing in neurological patients given the poor predictive value of some coeliac antibodies particularly IgG AGA. Secondly, immunologists should be encouraged to provide well-defined, reproducible diagnostic tests and for

these to be universally adopted amongst neurologists as a more acceptable marker of gluten sensitivity. It may be that TTG or its successor will be this test. Finally, more detailed investigation is required. Interpretation of population-based studies is difficult because occult sub-clinical CD occurs commonly and background prevalence needs to be accounted for. The inadequacy of previous studies from a methodological viewpoint needs to be addressed in future work on this subject. I suggest that this requires inter-disciplinary collaboration between neurologists, immunologists, gastroenterologists, geneticists and probably, most importantly, epidemiologists.

9.5 Areas for future study

Future studies would be increasingly powered by having large patient numbers, long term follow-up and possibly national or multinational registries of patients. Further large studies are required looking for the prevalence of neurological conditions in CD and DH, the role of auto-immunity, particularly in susceptible HLA groups, in neurological diseases seen with CD.

Randomised, controlled trials for treatment efficacy would be practically difficult. Studies with animal models of CD may provide important insights and this is certainly an exciting area for real-time study of this disease process in carefully controlled environments.

9.5.1 Epilepsy and coeliac disease

Whilst I have shown that a causative relationship between epilepsy and CD appears less likely, further study is recommended e.g. in the form of a case-control study to determine firstly a consistent observed association and secondly to demonstrate a plausible scientific link. It may be that certain

groups of patients are at increased risk such as patients with significant folate deficiency. Furthermore, even if no actual association exists, it would be important to identify a treatable cause of malabsorption because of potentially impaired drug absorption.

9.5.2 Ataxia and coeliac disease

More in-depth study is required into the intriguing association of gliadin positivity with ataxia (both idiopathic and hereditary). Ataxia, be it sensory or cerebellar, idiopathic or hereditary, has been widely investigated. However many questions still remain. This research has raised the possibility of an anti-spinal antibody which may contribute to the aetiology of a sensory ataxia. Positive gliadin antibodies, which are an unsatisfactory test in terms of sensitivity and specificity, are an intriguing and as yet unexplained finding. The presence of these in both hereditary and idiopathic cerebellar ataxias suggests a possible inflammatory aetiology. However it is well-known that these antibodies are raised in other autoimmune conditions such as diabetes and hypothyroidism. As such further study into the importance of this finding may prove to be fruitless. It may be of greater worth to accept that cerebellar ataxia has an inflammatory basis and direct further study towards exploring this mechanism from a different angle.

9.5.3 The role of trace elements

The neurological complications of CD, and indeed other enteric disease causing malabsorption, is a fascinating area for study as malabsorption may yet be proven to be of greater significance in this association than was previously realised. Although vitamin deficiency does not satisfactorily explain patients in whom no vitamin deficiency is found (Ward, Murphy et al. 1985; Lu,

Thompson et al. 1986) or in whom vitamin replacement has no effect (Bhatia, Brown et al. 1995), this possibility should still be carefully considered in CD patients who develop neurological illness. Whilst the role of some vitamins has been well-researched and well-documented, the role of numerous others is less clearly understood and moreover testing for these is often not routinely available. As yet, no studies have effectively addressed the role of trace vitamin deficiency (e.g. niacin, riboflavin and thiamine) in the development of neurological complications.

9.5.4 Ongoing research generated by this study

As demonstrated in part of this thesis, use of such databases such as the Coeliac Register set up by Dr Holmes in Derby, spanning many years and including large numbers of patients, is also an extremely informative source albeit flawed by potential observer bias. The General Practice Research Database (GPRD) is a computerised database of anonymised patient information obtained from General Practitioners including demographics, medical diagnosis, prescriptions, referrals etc. Data is contributed by numerous GP practices in the UK and currently consists of information on approximately 3 million patients (approximately 5% of the UK population). Collaboration is ongoing with colleagues in the Public Health Department at Nottingham University to examine the GPRD to carry out a historical cohort study.

I aim to carry out further characterisation of the spinal antibody found in the DH cohort. Early immunohistochemical data suggests binding to dorsal column neurones from serum of these patients however this finding requires further confirmation.

9.5.5 Ongoing research worldwide

Outcome of the NINDS study (which is currently recruiting patients) will be of great interest (http://clinicaltrials.gov). The investigators aim to screen patients with cerebellar ataxia for evidence of gluten sensitivity. Those with positive antibodies will then be commenced on GFD and monitored 3 monthly for 12 months both for compliance to GFD and evaluation of ataxia. This is an open label controlled trial of GFD in patients with gluten sensitivity and cerebellar ataxia. Their controls will be patients with genetically-confirmed cerebellar ataxia e.g. SCA 1, 2, 3, 6, 7 and Friedrich's ataxia or cerebellar ataxia from any other known cause e.g. alcohol, cerebrovascular disease). They also aim to have age and sex-matched normal subjects.

9.6 Interpretation of results

9.6.1 No evidence for gluten neurotoxicity

The notion of gluten being neurotoxic is an attractive one. A GFD is a relatively simple and seemingly inexpensive treatment option. Moreover, with increased ease of screening for CD, the diagnosis would appear to be relatively straightforward. However the current study found no evidence to support the hypothesis that gluten sensitive enteropathy occurs with any greater frequency in patients with idiopathic neurological syndromes, namely multiple sclerosis, ataxia and neuropathy.

It must firstly be stressed that associations do not prove causality and attributing a cure for patients with so-called gluten-related neurological illness may be detrimental. From an individual point of view, a strict GFD may be unpleasant and troublesome. Coeliac patients with few or no symptoms identified through screening will have to accept the burden of life-long

treatment in return for ill-defined benefits. Overall patients with CD have a two-fold increase in mortality compared to the general population (Logan, Rifkind et al. 1989). However, prognosis in patients who are asymptomatic is not known. Moreover there is a body of evidence that suggests some protective effects of CD in terms of lower mortality from cardiovascular disease, lower blood pressure and cholesterol levels than the general population (Green and Wollaeger 1960; Vuoristo and Miettinen 1985).

Our understanding of autoimmune pathogenesis and the contribution of genetic and environmental factors is limited. We are still in an embryonic phase of understanding antineuronal pathology. Whilst we assume that antineuronal antibodies are pathogenic this has still not been robustly proven (although passive transfer of antibodies does induce disease features and reduction of antibody titres is often therapeutic). Genetic mechanisms of pathogenesis are also incompletely understood. It is far more likely that the neurological complications of CD are a combination of disease processes which may have the commonality of a single antibody (AGA) on serological testing as an epiphenomenon rather than a causative factor.

9.6.2 Gluten ataxia not analogous to dermatitis herpetiformis

It has been claimed that “gluten ataxia is a manifestation of gluten sensitivity ….. analogous to the example of dermatitis herpetiformis, from which it is apparent that the gut is not the sole protagonist in this disease” (Hadjivassiliou, Grunewald et al. 2003). However AGA seropositivity does not equate to gluten sensitivity, a term that should be reserved for cases in which there is unequivocal, objective evidence of benefit with a GFD.

Using DH as typifying a gluten-sensitive illness in which a non-gastrointestinal syndrome predominates is problematic. Gluten ataxia and DH are not analogous in this regard. Although less than 10% of DH patients have gastrointestinal symptoms, they are all said to have a gluten-sensitive enteropathy (Reunala 1998). Patients with a genetic susceptibility to gluten may have no intestinal abnormalities on small bowel biopsy but true latent CD is uncommon.

DH and CD are both strongly-associated with HLA DQ and with no genetic differences to explain the 2 phenotypes. DH is not fully understood but what evidence exists suggests precipitates of IgA in the papillary dermis containing epidermal transglutaminase. Epidermal and tissue transglutaminases in DH and |CD respectively are highly homologous. Cross-reactivity of the two antibodies may explain why patients with either DH or CD will have antibodies to both these enzymes but in DH the skin manifestation occurs because epidermal transglutaminase antibodies produced are of high avidity and affinity (Karpati 2004). DH is a very specific disease process with very specific, increasingly better-characterised immune mechanisms, none of which are robustly demonstrated in ‘gluten ataxia’.

9.6.3 Is screening worthwhile?

The criteria for mass screening are: a potentially serious condition with significant morbidity, difficult early clinical detection, availability of a sensitive, simple and cheap screening test and availability of an effective therapy. There is insufficient evidence to support mass screening for gluten sensitivity in unselected patient groups because uncertainty remains regarding the natural history of the disease. Specifically we need a clearer understanding of the

outcome in affected individuals who are essentially asymptomatic. Knowledge of lead-time duration (interval between detection and time at which diagnosis would have been made without screening) is crucial to rationalise the need for screening. At present, coeliac specialists advocate case finding i.e. remaining alert to the possibility of this diagnosis in patients with suggestive signs, symptoms or haematological/biochemical indices.

On the basis of the available literature and work derived from this thesis, routine screening for gluten sensitivity in patients with neurological illness is not recommended unless there are signs and symptoms to suggest an underlying diagnosis. However, clinicians should remain vigilant for malabsorption which is potentially treatable and carry out careful screening for deficiency of vitamins and trace elements in patients known to have CD who present with neurological disease.

9.7 Conclusion

Disease reporting both from local experience as well as nationally has not suggested an association between gluten sensitivity and neurological disease. This research found no evidence of excessive neurological complications in patients with gluten sensitivity (DH and CD), nor evidence of increased gluten sensitivity in patients with neurological illness. One intriguing finding has been the demonstration of a novel anti-spinal antibody the significance of which remains to be determined. One further important finding demonstrated was that AGA (particularly of IgG isotype) is flawed by poor sensitivity, specificity and predictive value.

There continue to be conflicting reports regarding the nature of the association between gluten sensitivity and neurological disease. This work does not support this association. AGA positivity occurs in numerous neurological conditions but this does not equate with gluten neurotoxicity. The role of gliadin antibodies in neurological disease warrants further investigation. In view of the ongoing controversy, we must await further, scientifically-robust data to clarify this issue. However, it is hoped that this research has contributed to the interpretation of clinical and immunological data with regard to patients with neurological disease and possible gluten sensitivity.

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