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Title

Transglutaminase 6 antibodies in gluten neuropathy

Running title

TG6 in gluten neuropathy

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Marios Hadjivassiliou: drafting/revising the manuscript, study concept and design, data collection, accepts responsibility for conduct of research and final approval.

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Disclosures

Panagiotis Zis, Dasappaiah Ganesh Rao, Ptolemaios Georgios Sarrigiannis, Pascale Aeschlimann, David Sanders Richard A Grünewald and Marios Hadjivassiliou have nothing to disclose.

Daniel Aeschlimann serves as a scientific advisor/collaborator to Zedira (without financial incentives) but receives royalties from Zedira for patents.

Conflict of interest

None

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ABSTRACT (236 words)

Background:

TG6 antibodies have been shown to be a marker of gluten ataxia but their presence in the context of other neurological manifestations of gluten sensitivity has not been explored. We investigated the presence of TG6 antibodies in gluten neuropathy (GN), defined as an otherwise idiopathic peripheral neuropathy associated with serological markers of gluten sensitivity (one or more of antigliadin IgG and/or IgA, endomysial and transglutaminase-2 antibodies)

Methods:

This was a cross-sectional study conducted at the Sheffield Institute of Gluten Related Diseases, Royal Hallamshire Hospital, Sheffield, UK. Blood samples were collected whilst the patients were on a gluten containing diet. Duodenal biopsies were performed to establish the presence of enteropathy.

Results:

Twenty-eight patients were recruited (mean age 62.5 ± 13.7 years). Fifteen (53.6%) had sensory ganglionopathy, 12 (42.9%) had symmetrical axonal neuropathy and 1 had mononeuritis multiplex. The prevalence of TG6 antibodies was 14 of 28 (50%) compared to 4% in the healthy population. TG6 antibodies were found in 5/15 (33.3%) patients with sensory ganglionopathy and in 8/12 (66.7%) with symmetrical axonal neuropathy. Twenty-four patients underwent duodenal biopsy 11 (45.8%) of which had enteropathy. The prevalence of TG6 was not significantly different when comparing those with or without enteropathy.

Conclusions:

We found a high prevalence of antibodies against TG6 in patients with GN. This suggests that TG6 involvement is not confined to the central nervous system. The role of transglutaminase 6 in peripheral nerve function remains to be determined but TG6 antibodies may be helpful in the diagnosis of GN.

INTRODUCTION

Gluten neuropathy is the second commonest neurological manifestation of gluten sensitivity [1]. It is defined as an otherwise idiopathic peripheral neuropathy associated with serological markers of gluten sensitivity (one or more of antigliadin IgG and/or IgA, endomysial and transglutaminase-2 antibodies) [2]. The commonest type of gluten neuropathy is symmetrical sensorimotor axonal peripheral neuropathy [1]. Sensory ganglionopathy, an asymmetrical pure sensory neuropathy is the second commonest form of neuropathy seen in the context of gluten sensitivity [3]. This type of neuropathy exclusively affects the dorsal root ganglia and the commonest causes apart from gluten sensitivity include Sjogren's syndrome and paraneoplastic neurological syndromes. Much more rare types of neuropathies such as mononeuritis multiplex and small fiber neuropathy have also been described in the context of gluten sensitivity [4,5].

Neurological manifestations of gluten sensitivity might not only occur in the context of coeliac disease (CD) but also in non-coeliac gluten sensitivity (NCGS) [6]. Although some markers such as anti-tissue transglutaminase (TG2) and anti-endomysium antibodies are sensitive and specific in diagnosing CD, such antibodies will usually be absent in patients with NCGS [6]. Anti-gliadin antibodies, however, are indicators of NCGS as up to 50% of such patients presenting to gastroenterologists have detectable circulating levels of anti-gliadin antibodies [7].

The discovery of TG6, a transglutaminase primarily expressed in neural tissue, has offered an opportunity for the development of a more specific marker for neurologic manifestations of gluten sensitivity as well as contributing to some clarification of the pathophysiology of neurological involvement [8, 9]. Like TG2 in Coeliac Disease (CD) and TG3 in dermatitis herpetiformis, TG6 is likely to be the autoantigen in gluten related diseases where the primary manifestation is neurological [1]. TG2, TG3 and TG6 share 65% homology, they are capable of deamidating gluten peptides and antibodies against TG2, TG3 and TG6 are eliminated with strict adherence to a gluten-free diet [10]. TG6 antibodies are present in 38% of patients with newly

diagnosed CD but in only 4% of normal subjects [6, 10]. Moreover, the prevalence of TG6 in patients with gluten ataxia is 73% [10]. We do not know if TG6 is confined to the central nervous system or if it may also be present in the peripheral nervous system (including gut neural plexus) and thus wanted to investigate its presence in patients whose neurological manifestation appears to be primarily peripheral (i.e. peripheral neuropathy).

METHODS

Patient selection. This was a cross-sectional study conducted at the Sheffield Institute of Gluten Related Diseases (SIGReD). Patients were recruited from the gluten/neurology clinic based at the Royal Hallamshire Hospital (Sheffield, UK). All patients presented with neurological symptoms suggestive of peripheral neuropathy, which led to them being investigated for the possibility of gluten sensitivity and coeliac disease. Patients were diagnosed with gluten neuropathy, when having serological evidence of gluten sensitivity at baseline serology (one or more of anti-gliadin IgG and/or IgA, endomysial and transglutaminase-2 antibodies) and clinical evidence of neuropathy that was further confirmed with nerve conduction tests. The clinical presentation was typical of peripheral neuropathy (distal or patchy sensory loss with areflexia) without any suggestion of cerebellar ataxia.

Electrophysiology. Extensive neurophysiological assessment was performed in all patients and included nerve conduction studies of median (motor and sensory), ulnar (motor and sensory), superficial radial (sensory), superficial peroneal (sensory), common peroneal (motor), sural (sensory) and tibial (motor) nerves on both sides. Based on the electrophysiological findings the patients were diagnosed with symmetrical sensori-motor axonal neuropathy where amplitudes were symmetrically attenuated and involved both motor and sensory nerves. Patients were diagnosed with sensory ganglionopathy when only sensory fibers were affected in an asymmetrical fashion or where no sensory responses were elicited by any sensory nerve with motor responses being normal [11]. Finally, patients with involvement of

sensory and motor fibers of specific nerves, sparing others, were diagnosed with mononeuritis multiplex.

Standard protocol approvals, registrations, and patient consents. The South Yorkshire Research Ethics Committee approved the study, and all patients provided written informed consent.

Serologic testing. Serological testing was performed on baseline serum collected before the introduction of a gluten-free diet. Determination of anti-TG6 IgA and IgG was done using in house ELISAs. The methodology has been described in detail previously [12]. Human leukocyte antigen (HLA) typing was performed by the regional blood-transfusion service.

Endoscopy. All patients were referred for an endoscopy and duodenal biopsy to establish the presence of enteropathy. All biopsies were histologically assessed by an experienced pathologist for evidence of enteropathy (triad of villous atrophy, crypt hyperplasia, and increase in intraepithelial lymphocytes).

Statistical analysis. A database was developed using the statistical software package SPSS (version 23.0 for Macintosh). Descriptive statistics were examined for each variable. Statistical comparisons were performed between the patients with and without enteropathy concerning clinical characteristics and presence of TG6 antibodies. We also compared the TG6 positive patients with the TG6 negative patients regarding the clinical characteristics, electrophysiological type of neuropathy, presence of enteropathy and HLA type. Categorical variables were compared using the chi square test and non-normally distributed continuous variables were compared using the Mann-Whitney U test. Level of significance was set at 95% confidence level.

RESULTS

Clinical characteristics. We recruited 28 patients with gluten neuropathy (75% males). Mean age at onset of the symptoms was 60.3 ± 13.8 and mean age at diagnosis was 62.5 ± 13.7 years. Based on the neurophysiological assessments, 15 patients (53.6%) were found to have a sensory ganglionopathy (SG), 12 patients (42.9%) had symmetrical axonal sensorimotor neuropathy and 1 patient had mononeuritis multiplex.

Prevalence of TG6 antibodies in the study group. The prevalence of TG6 antibodies was 14 of 28 (50%). TG6 antibodies were present in 5/15 (33.3%) in SG and in 8/12 (66.7%) in patients with symmetrical axonal neuropathy. The patient with the mononeuritis multiplex was also positive for TG6 antibodies. No statistically significant difference regarding presence of TG6 antibodies between the different neuropathy groups was observed ($p=0.115$).

Prevalence of TG2 antibodies in the study group. The prevalence of TG2 antibodies was 11 of 23 (47.8%). TG2 antibodies were present in 4/11 (36.4%) in SG and in 6/11 (54.5%) in patients with symmetrical axonal neuropathy. The patient with the mononeuritis multiplex was also positive for TG2 antibodies. The overall sub-group figures are very similar to the prevalence of TG6 antibodies mentioned above. However, TG6 antibodies were present in 5/12 (41.7%) patients who were negative for TG2 antibodies.

Endoscopy. Not all of the patients agreed to a duodenal biopsy. Eleven of 24 (45.8%) patients who did have an endoscopy and duodenal biopsy had enteropathy (9 coeliac disease, 2 increased intraepithelial lymphocytes). Patients with and without enteropathy did not differ statistically regarding gender, age and type of neuropathy. The prevalence of TG6 was not significantly different between the two groups, although there was a tendency for TG6 to be more prevalent in those patients with enteropathy (63.6% with and 38.5% without enteropathy, $p=0.219$)

HLA type. HLA typing was performed in 26 patients. Of them, 15 patients (57.7%) had the DQ2 type, 1 patient (3.8%) had the DQ8 type, 8 patients (30.8%) had the DQ1 type (30.8%) and 2 patients (7.7%) had another HLA type.

Table 1 summarizes the clinical, neurophysiological and serological characteristics of our study population.

TG6 positive versus TG6 negative patients.

No statistically significant differences between the TG6 positive and the TG6 negative patients were found regarding age, gender, type of neuropathy and HLA type. However there was a tendency for the TG6 positive group of GN patients to have symmetrical axonal sensorimotor neuropathy rather than sensory ganglionopathy. Table 2 summarizes the clinical characteristics of the TG6 positive and the TG6 negative groups.

DISCUSSION

The link between peripheral neuropathy and CD has now been well established with large epidemiological studies demonstrating a 2.5-fold increased risk of neuropathy for patients with CD [13]. This is the first study to examine the presence of autoantibodies against TG6 in what is the second commonest neurological manifestation of gluten sensitivity and CD. We found that the prevalence of TG6 antibodies is 50% in patients with gluten neuropathy, before embarking on a gluten free diet. This percentage is significantly higher to the prevalence of TG6 antibodies in healthy controls, which is only 4% [10].

TG6, like TG2 and TG3 belongs to a family of enzymes that covalently crosslink or modify proteins through transamidation, deamidation or esterification of a peptide-bound glutamine residue [14]. Gluten proteins (from wheat, barley and rye), the immunological trigger of GRDs, are glutamine rich donor substrates amenable to

deamidation. Deamidation of gluten peptides enhances binding with disease-relevant HLAs and thereby enhances presentation, leading to the development of gluten-specific CD4⁺ T cells resulting in inflammation leading to the typical enteropathy [15]. Whilst TG2 has been shown to be the autoantigen in CD [16] and epidermal transglutaminase TG3 has been shown to be the autoantigen in DH [17], antibodies against TG6, a primarily brain expressed transglutaminase have been shown to be present in patients with GA [10, 12]. In a previous study from our group and using the same methodology, 73% of patients with gluten ataxia were positive for TG6 antibodies [10]. Similar to TG2 and TG3 antibodies, the production of TG6 antibodies is gluten-dependent [10]. All three TG isozymes (TG2, TG3, TG6) for which autoantibodies have been described, can form thioester-linked complexes with gluten peptides which are thought to be responsible for the B cell response to TG isozymes [14, 18]. Pathogenicity of such circulating anti-TG antibodies in terms of leading to extraintestinal disease has been demonstrated in animal models [19, 20].

In this study we wanted to explore the role (if any) of TG6 antibodies in the second commonest neurological manifestation of gluten sensitivity, which is gluten neuropathy [1]. We do not know if TG6 is also present in peripheral nervous tissue, which of course includes the gut neural plexus. This study demonstrates a high prevalence of TG6 antibodies (50%) in patients with gluten neuropathy. This is irrespective of the presence or not of enteropathy, a similar finding to what we had found in patients with gluten ataxia. There are two possible ways of interpreting this finding. Firstly, it is possible that those patients with neuropathy and positive TG6 may already have some degree of subclinical cerebellar involvement. Whilst this is a possibility, all of our patients with gluten sensitivity, presenting with neurological dysfunction routinely undergo MR spectroscopy of the cerebellum to look for any cerebellar dysfunction, even in the absence of overt ataxia [21]. If they have cerebellar involvement they are more likely to be labeled as having gluten ataxia. We cannot exclude the possibility that these patients may later go on to develop ataxia as well. Indeed it has recently been shown that TG6 positivity correlates with duration of gluten exposure [22]. The second possibility is that TG6 is also found in the peripheral nervous system including the neural plexus of the gut. There is no

doubt, however that antibodies to TG6 are a marker of neurological involvement. Amongst patients with CD the prevalence of TG6 in those presenting with classic gastrointestinal symptoms was 38% vs 67% in those presenting with neurological symptoms [6]. At the same time the prevalence of TG6 in patients with non-coeliac gluten sensitivity presenting with neurological dysfunction was similar to those presenting with neurological dysfunction who also had enteropathy 60% vs 67% [6]. Finally it is worth noting that patients with gluten neuropathy present at a later age than patients with gluten ataxia 60.3 vs 52 years.

As it is often the case in autoimmune disorders [23], there is a female predominance in CD as well [24]. Our study population, however, included more male than female patients. Bai et al. showed indirectly that men show greater malabsorption than females [24]. Although malabsorption, and particularly vitamin B12 deficiency is a known risk factor for neuropathy, our study population by definition had no evidence of such deficiency. Therefore, the most likely explanation for this discrepancy in male to female ratio is that our study sample was small but also that in NCGS such female predominance is also not observed

More than 95% of patients with CD share the HLA DQ2 or DQ8 haplotype [25]. In our study population 62% of patients with gluten neuropathy shared those HLA types. This may be explained by the fact that gluten neuropathy per definition does not require the patient to have enteropathy.

We acknowledge as a limitation of our study the small number of patients investigated and the need for a prospective evaluation of patients with axonal neuropathy and sensory ganglionopathy for the presence of TG6 antibodies with and without the presence of any of the other gluten related serological markers. However, the prevalence of antibodies against TG6 in patients with gluten neuropathy in this study is significantly high and suggests that the presence of such antibodies may help further in the clarification of the pathophysiology of the extraintestinal manifestations of gluten sensitivity as well as being helpful in the diagnosis of GN. Hopefully, the development of commercial kits for the detection of TG6 antibodies, which are currently lacking, will improve the diagnosis of GN.

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