Transglutaminase 6 Antibodies in the Serum of Patients With Amyotrophic Lateral Sclerosis

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IMPORTANCE Celiac disease is an autoimmune disorder triggered by gluten in genetically predisposed individuals. Gluten sensitivity can cause neurologic manifestations, such as ataxia or neuropathy, with or without gastrointestinal symptoms. Many patients with gluten ataxia produce antibodies toward the newly identified neuronal transglutaminase 6 (TG6). Two case reports described patients initially diagnosed with amyotrophic lateral sclerosis (ALS) and ultimately with celiac disease who improved with a strict gluten-free diet.

OBJECTIVE To evaluate the prevalence of celiac disease–related antibodies and HLA antigen alleles, as well as TG6 antibodies, in patients with ALS and healthy individuals serving as controls to determine whether a neurologic presentation of a gluten-related disorder mimicking ALS might occur in some patients.

DESIGN, SETTING, AND PARTICIPANTS In a case-control study conducted in an ALS tertiary center, we measured serum levels of total IgA antibodies, IgA antibodies to transglutaminase 2 (TG2) and endomysium, as well as IgA and IgG antibodies to deamidated gliadine peptide and TG6 and performed HLA antigen genotyping in 150 consecutive patients with ALS and 115 healthy volunteers of similar age and sex. Participants did not have any known autoimmune or gastroenterologic disorder and were not receiving any immunomodulatory medications. The study was conducted from July 1, 2010, to December 31, 2012.

MAIN OUTCOMES AND MEASURES Antibody levels and frequency of individuals with abnormal antibody values as well as frequency of HLA antigen alleles were compared between patient and control groups.

RESULTS All patients and control group participants were seronegative to IgA antibodies to TG2, endomysium, and deamidated gliadine peptide. Twenty-three patients (15.3%) were seropositive to TG6 IgA antibodies as opposed to only 5 controls (4.3%) (P = .004). The patients seropositive for TG6 showed a classic picture of ALS, similar to that of seronegative patients. Fifty patients and 20 controls were tested for celiac disease–specific HLA antigen alleles; 13 of 22 TG6 IgA seropositive individuals (59.1%) were seropositive for celiac disease–related alleles compared with 8 (28.6%) of the 28 seronegative individuals (P = .04). Mean (SD) levels of IgA antibodies to TG2 were 1.78 (0.73) in patients and 1.58 (0.68) in controls (normal, <10). In a subset of study participants, mean levels of deamidated gliadin peptide autoantibodies were 7.46 (6.92) in patients and 6.08 (3.90) in controls (normal, <16). Mean levels of IgA antibodies to TG6 were 29.3 (30.1) in patients and 21.0 (27.4) in controls (P = .02; normal, <26).

CONCLUSIONS AND RELEVANCE The data from this study indicate that, in certain cases, an ALS syndrome might be associated with autoimmunity and gluten sensitivity. Although the data are preliminary and need replication, gluten sensitivity is potentially treatable; therefore, this diagnostic challenge should not be overlooked.

Published online April 13, 2015.
Celiac disease (CD) is a chronic, small-intestine immune-mediated enteropathy triggered by exposure to dietary gluten in genetically predisposed individuals; the disease resolves with a strict gluten-free diet.\(^1,2\) The incidence of CD seems to increase in the Western population.\(^3\) Celiac disease has a prominent genetic susceptibility linkage to the HLA antigen class II region in white individuals, more than 90% of patients carry the DQ2 variant and most others carry DQ8 or both variants.\(^4,5\) However, the HLA-DQ2 haplotype is common (present in 30% of the white population),\(^6\) although only approximately 3% of these individuals will develop CD.\(^7\) Gluten proteins undergo deamidation by tissue transglutaminase (TG)2 in the intestinal mucosa, and these parts are presented on the HLA-DQ2/DQ8 as a foreign antigen. This process initiates the immune response.\(^8\) Tissue transglutaminase 2 is the primary autoantigen of CD, and TG2 autoantibody production, which is induced by gluten, is used as initial testing for CD. Serologic tests used for the diagnosis of CD are IgA TG2 antibodies, IgA antiendomysium (EMA), and deamidated gliadin peptide (DGP) antibodies.

Gluten can cause extraintestinal manifestations, such as dermatitis herpetiformis and neuropsychiatric disorders, mainly cerebellar (gluten ataxia) and peripheral neuropathy, even without intestinal involvement and CD-related antibodies; this condition is referred to as nonceliac gluten sensitivity (NCGS).\(^9,10\) Such patients typically have antibodies that react to a different TG isoenzyme: TG3 in dermatitis herpetiformis\(^11\) and TG6 in gluten ataxia.\(^12\)

Tissue transglutaminase 6 is primarily expressed in the brain.\(^13\) The prevalence of TG6 antibodies (IgA and/or IgG) was reported in 62% of patients with gluten ataxia and in 45% of those with CD without neurologic manifestations.\(^12\) The TG6 antibodies are sensitive and specific in gluten ataxia. The TG6 antibody titer is dependent on gluten ingestion.\(^14\) In addition, TG6 antibodies have been reported in gluten neuropathy,\(^15\) schizophrenia,\(^16\) cerebral palsy,\(^17\) and a rare neurologic syndrome associated with epilepsy and cerebral calcification.\(^18\) Other neurologic syndromes, such as epilepsy, encephalopathy, dementia, myopathy, myelopathy, multiple sclerosis, stiff-man syndrome, and myoclonic ataxia, have been rarely reported in association with gluten sensitivity.\(^5,18\)

Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease with unknown pathogenesis.\(^19\) The diagnosis of ALS is entirely clinical and is determined by finding signs of involvement of upper and lower motor neurons after exclusion of alternative diagnoses. There are no biomarkers for the diagnosis of ALS.

Two independent cases of men with an initial diagnosis of ALS that ultimately was identified as CD have been reported.\(^20,21\) In both patients, the initial diagnosis was based on clinical symptoms as well as on the results of magnetic resonance imaging and electromyography. The diagnosis of CD was established by celiac disease-related antibodies and duodenal biopsy results. With a strict gluten-free diet, both patients experienced improvement of the symptoms.

The objective of this study was to examine the presence of gluten sensitivity-related antibodies in patients with ALS to evaluate whether a neurologic presentation of gluten sensitivity mimicking ALS might occur in some patients. In such a presentation, a gluten-free diet might improve the symptoms of this subset of patients.

### Methods

#### Study Population

This case-control study included 150 patients with definite or probable ALS according to the revised El Escorial criteria\(^22\) who were monitored at the ALS clinic at Tel Aviv Medical Center in Israel. A total of 115 healthy volunteers of similar age and sex were used as a control group. Individuals with chronic gastrointestinal symptoms, any autoimmune disease, or immunomodulatory treatment were excluded. All individuals provided written informed consent to participate in this study that was approved by the local institutional review board at our center. There was no financial compensation. The study was conducted from July 1, 2010, to December 31, 2012.

Demographic (age and sex) and clinical (age at disease onset, presentation form at onset, and survival) data were collected. Survival was defined as number of months from first symptom until occurrence of tracheostomy or death. Present or past gastrointestinal symptoms (diarrhea, constipation, and abdominal pain) and a familial history of neurologic and gastrointestinal diseases were recorded.

#### Serologic Testing

Conventional celiac detection was performed on serum samples that were frozen (−80°C) for a maximum of 4 months before the test. The total IgA level was determined by a nephelometric BN2 system (N Antiserum to Human IgA; Siemens). The IgA antibodies to TG2 were measured by enzyme-linked immunosorbent assay (Anti Tissuetransglutaminase IgA; Orgentec) and IgA/IgG antibodies to EMA in the serum by immunofluorescence kit (Immuglo EMA Endomysial Antibody IFA; Immco Diagnostics). Measurements of human neuronal TG6 IgA and IgG antibodies were performed by enzyme-linked immunosorbent assay (Transglutaminase-6 Ab [IgA Elisa; Zedira]. Cutoff scores were calculated as the mean of the control group plus 2 SDs and were set at greater than 42 U/mL for IgA and >43 U/mL for IgG. Antibodies (IgA/IgG) against DGP in serum were measured in 60 patients (including all those seropositive to TG6 antibodies) and 25 controls by enzyme-linked immunosorbent assay (Celicheck new generation; Aesku).

#### HLA Antigen Typing

Genotyping of HLA antigen for CD-associated alleles was performed in all 23 patients who were seropositive to IgA TG6 antibodies, in 27 patients negative for these antibodies, and in 20 controls. The following alleles were examined: DQA1*0501 and *0505 and DQB1*0201, *0202, and *0302 (HLA-Ready Gene kit for CD; Inno-train Diagnostik).

#### Statistical Analysis

The Pearson χ² test or Fisher exact was used to compare the groups of patients and controls with respect to sex, TG6 anti-
body positivity (percentage), and HLA antigen and the groups of patients seropositive or seronegative to IgA TG6 antibodies with respect to sex, bulbar form, and family history of ALS. The age and TG6 IgA/IgG antibody concentrations in patients and controls, as well as the age at onset in the 2 groups of patients, were compared using the 2-sample t test.

The Kaplan-Meier method was used to compute product-limit estimates of the survival distribution function of mortality for each of the patient groups (positive or negative to IgA TG6 antibodies). The log-rank test was used to compare survival curves of the 2 groups. Statistical analysis was performed using SAS for Windows, version 9.2 (SAS Institute Inc).

Results

Study Population
A total of 150 patients (men, 99 [66.0%]; mean [SD] age, 61.7 [12.3] years) and 115 controls (men, 86 [74.8%]; age, 61.05 [11.5] years) participated in this study (Table 1). The patient population had a mean age at disease onset of 59.7 [12.4] years and survived for 38.2 [20.0] months. A total of 27.8% of the patients had a bulbar onset of ALS, and 63.3% had received a tracheostomy or were dead at the time of data analysis. The mean disease duration from symptom onset to time of blood test was 19.8 [15.2] months.

CD-Related Antibodies
All patients and controls had levels of total IgA within the reference range and were negative for IgA TG2 antibodies (mean levels, 1.78 [0.73] in patients and 1.58 [0.68] in controls; normal, <16). The 1 patient who was seropositive to DGP was seropositive also for TG6 IgA; this woman died 12 months after disease onset.

TG6 Autoantibodies
IgA Antibodies
Twenty-three of 150 patients (15.3%) were seropositive to TG6 IgA antibodies compared with only 5 of 115 controls (4.3%) (P = .004). Three additional patients had borderline levels of IgA TG6 antibodies (42 U/mL). The mean (SD) IgA TG6 antibody concentration (29.3 [30.1] U/mL) in the patient group was significantly higher than that in the control group (21.0 [27.4] U/mL) (P = .02). Demographic and serologic data of patients and controls are reported in Table 1.

Patients seropositive for TG6 IgA showed a classic picture of ALS: 73.9% men, mean (SD) age of 56.3 (13.9) years at disease onset, 4 individuals (17.4%) with bulbar onset, median survival of 56 months, and 17.4% with a family history of ALS (Table 2). Patients had a typical rate of disease progression. Eight patients received a tracheostomy or died a mean (SD) of 24.5 (25) months (range, 7-86 months) after disease onset. The IgA-seronegative TG6 patients showed a similar clinical presentation (Table 2).

IgG Antibodies
The TG6 IgG antibodies were measured in 100 patients and 81 controls. Three individuals in each group were seropositive for TG6 IgG antibodies. Two of these participants were also seropositive for TG6 IgA antibodies. All 3 of the controls who were seropositive for TG6 IgG antibodies were negative for TG6 IgA antibody. The mean TG6 IgG antibody concentration was similar in both groups (14.8 [11.9] U/mL in the patients and 14.5 [14.0] U/mL in the controls).

HLA Antigen Typing
Fifty patients and 20 controls were tested for association with CD-specific HLA antigen alleles. The frequency of the different combinations of the tested DQA1* and DQB1* alleles was similar in patients and controls (Table 1). However, 13 of the 22 patients (59.1%) who were seropositive for IgA TG6 antibodies were seropositive to both DQA1* and DQB1* alleles. This frequency is significantly higher (P = .04) compared with 8 of 28 patients (28.6%) without IgA TG6 antibodies.
Discussion

Occurrence of TG6 Autoantibodies in ALS
The main result of the present study is that 15.3% of the tested patients with ALS were seropositive to IgA TG6 antibodies, although all 150 of these patients were seronegative to CD-related antibodies. This prevalence is significantly higher compared with only 4.3% of the control individuals with seropositive IgA TG6 antibodies. The concentration of TG6 IgA antibodies was also significantly higher in patients with ALS compared with the controls.

The presence of TG6 autoantibodies in patients with ALS has not been reported previously. However, TG6 antibodies have been detected in NCGS neurologic syndromes and have been proved able to identify gluten sensitivity in patients with ataxia who lacked symptoms or other serologic markers that may indicate gluten sensitivity, although their significance as markers of gluten-triggered neurologic disorders is controversial.

All patients with ALS in this study were seronegative for TG2, EMA, and DGP antibodies (except 1 patient, who was seropositive to DGP antibodies), clearly demonstrating that none of them had CD, but these tests do not exclude NCGS. The patient who was seropositive for DGP antibodies had a normal level of total IgA and was seronegative for TG2 and EMA antibodies, which means that she did not have CD, and the positive result of DGP antibody testing has to be attributed to another factor (though false-positive) Further evaluation for the presence of TG6 autoantibodies detected TG6 IgA antibodies in 15.3% of the tested patients, indicating a possible gluten sensitivity-related neurologic disorder in this group of patients with ALS. These results resemble the data reported in a group of patients with cerebellar ataxia who were seropositive for TG6 antibodies (IgG and IgA), although they were seronegative for antigliadin, TG2 antibodies, and antiendomysium. One control individual in our study was seropositive for TG2 and DGP antibodies, which clearly indicates the presence of asymptomatic CD in some healthy people. This control participant was seronegative for TG6 antibodies.

Association of TG6 Autoantibodies and HLA Antigen Class II Alleles
Thirteen of 22 patients with ALS (59.1%) with circulating IgA antibodies to TG6 were seropositive for both DQA1* and DQB1* alleles (carry HLA-DQ2) and 3 of 22 patients (13.6%) were seropositive only for DQB1 alleles (carry HLA-DQ8 or HLA-DQ2) and 3 of 22 (13.6%) were seropositive only for DQA1 alleles. In total, 19 of 22 of our patients (86.4%) who were seropositive for TG6 autoantibodies carry the HLA antigen susceptibility alleles for CD. The identification of HLA-DQ2 (or the half heterodimer DQBl*02) and DQ8 (DQB1*0302) along with seropositive TG6 antibody strengthens the possibility that gluten is involved in the pathologic mechanism in this subset of patients with ALS.

However, 13.6% (3 of 22) of the patients with ALS in this study who were seropositive for TG6 autoantibodies did not carry any of the tested DQA1* or DQB1* alleles. The absence of HLA-DQ2 or HLA-DQ8 is used clinically as an exclusion test for the presence or future development of CD. Unlike TG2, TG6 may catalyze a gluten-related reaction that is independent of the presence of HLA-DQ2/DQ8.

Clinical Presentation in Patients With or Without TG6 Autoantibodies
Patients seropositive for TG6 IgA antibodies showed a clinical presentation similar to that of patients negative for these antibodies regarding age at disease onset, proportion of bulbar cases, and sex distribution, much like the presentation of patients with schizophrenia. This similarity opposes the reported relatively early onset of disease in patients with gluten ataxia who are seropositive for TG6 IgA antibodies.

Production of TG6 autoantibodies was not significantly associated with a familial history of ALS. There was a trend toward longer survival in patients who were seropositive for TG6 IgA antibodies, but the association did not reach statistical significance. The similar clinical presentation may suggest that the autoimmune response mediated by TG6 autoantibodies leads to a common final pathologic pathway as the non-immune-mediated disease.

Anti-TG6 Autoantibodies as a Possible Biomarker for an ALS Syndrome
In contrast to IgA, IgG antibodies to TG6 did not differ significantly in prevalence or in concentration from healthy individuals, which shows that TG6 IgA, but not IgG, antibodies are seemingly useful in identifying patients with ALS who have gluten sensitivity. This finding is in concordance with Guan et al who determined that TG6 IgA antibodies, but not TG6 IgG antibodies, seem more useful for diagnosing gluten ataxia. The finding is also similar to that of Cascella et al, who suggested TG6 IgA antibodies as a useful biomarker for schizophrenia associated with gluten sensitivity. In the patients with ALS, TG6 IgA autoantibodies might constitute a marker that aids the identification of patients with gluten-dependent, autoimmune-based neurologic dysfunction.

Role of TG6 Autoantibodies in the Neuronal Damage in ALS
The role of TG6 autoantibodies in neuronal damage is unclear. Gluten may induce a primary autoimmune response, or TG6 antibodies may be a secondary effect of neuronal damage rather than being pathogenic. Similar to TG2 and TG3, TG6 can specifically deamidate gluten T-cell epitopes. In addition, TG6 has the ability to form a complex with gluten peptides through thioester linkage and is able to form isopeptide-linked complexes. These findings support a functional role for TG6 antibodies in an autoimmune mechanism triggered by gluten. In one study, cerebellar IgA deposits that contained TG6 were identified in postmortem tissue from patients with gluten ataxia. Isolated TG2, TG3, and TG6 antibodies cross-reactive single-chain variable fragments caused ataxia-like deficits when injected intraventricularly in mice.

In adult mice, TG6 is primarily and abundantly expressed in neurons across the central nervous system, including spinal motor neurons, and TG6 expression is associated with neurogenesis during central nervous system development and in regions

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associated with motor function.\textsuperscript{13-27} The gene encoding TG6 (TGM6; NM_198994 and NM_001254734) has recently been identified as a novel causative gene of one form of spinocerebellar ataxia.\textsuperscript{28-29} Taken together, these findings provide increasing evidence that TG6 may play an important role in motor control and that autoantibodies to TG6 might interfere with its action and be involved in the pathogenesis of neurodegenerative diseases. Detection of autoantibodies to TG6 has been recently suggested (patent application number: 20130196336, August 2013) as useful for the diagnosis of a gluten-related autoimmune disorder that is primarily neurologic or is characterized by prominent neurologic dysfunction.

**Role of TG2 in the Pathogenesis of ALS**

Mutant superoxide dismutase 1 (SOD1) is implicated in the pathogenesis of familial ALS, although the molecular basis of SOD1 oligomerization is not yet known. The cerebrospinal fluid of patients with ALS showed elevated levels of TG2 at the initial stage of the disease and decreased levels at the late stage of ALS.\textsuperscript{30} It was demonstrated recently\textsuperscript{31} that TG2 is involved in the aberrant assembly of misfolded SOD1 proteins, which contributes to neuroinflammation and disease progression in a mouse model of ALS. Those authors suggested a novel role for TG2 in SOD1 oligomer-mediated neuroinflammation and disease progression as well as in the intracellular aggregation of misfolded SOD1 in ALS. Our data suggest that autoimmune mechanisms involving transglutaminases may contribute to the pathogenesis of ALS.

**Association of ALS With Autoimmune Diseases**

It has been recently reported\textsuperscript{32} that patients with ALS had a 15\% overall excess risk of autoimmune diseases, with a 1.57 relative risk of prior CD. These results support an immunologic component and, specifically, an influence of gluten-related autoimmune processes to the pathogenesis of ALS.\textsuperscript{33} However, a Swedish study\textsuperscript{34} failed to show an excess of ALS cases among patients with CD. As opposed to these studies, our study does not refer to CD but to a possible association of ALS with NCGS. To our knowledge, this association has not been previously described.

The population of patients with ALS is heterogeneous, and an autoimmune mechanism could mediate the pathologic mechanism in a specific subgroup of patients. The inability to identify specific subgroups might be one of the explanations for the lack of effectiveness of immunosuppressive therapies in ALS.

**Gluten-Free Diet in Patients With Primary Neurologic Dysfunction**

Patients who produce TG6 antibodies may benefit from a gluten-free diet since it has been shown\textsuperscript{14} that TG6 antibodies are gluten dependent, although this dependence is controversial.\textsuperscript{23} In patients diagnosed with gluten ataxia or gluten neuropathy, a strict gluten-free diet is sometimes followed by improvement or stabilization of neurologic symptoms.\textsuperscript{10,35,36} In 2 case reports\textsuperscript{20,21} of CD mimicking ALS, the patients’ conditions improved with use of a strict gluten-free diet that was initiated 6 and 12 months after disease onset. The clinical response to a gluten-free diet depends on the duration of disease, which is probably the main factor in the irreversible damage to neurons before the start of the diet.\textsuperscript{5,37} Patients with long-lasting neurologic symptoms (>1 year) may not benefit from dietary removal of gluten.\textsuperscript{37} All 6 of our TG6-seropositive patients who remained alive without tracheostomy during the study had a prolonged disease course, with more than 3 years since disease onset. However, they were offered a gluten-free diet in light of the results presented here. Because the number of patients is small and the irreversible neuronal damage is presumably very advanced, we assume that we will not be able to draw any conclusions regarding therapy from this cohort.

**Conclusions**

Our study suggests that an ALS syndrome related to gluten sensitivity may occur in a subgroup of patients and that TG6 IgA autoantibodies may be a marker for identifying gluten-sensitive patients. Replication of our findings from other patient cohorts is needed to assess the reliability and clinical relevance of the findings.

In early identified cases of ALS associated with gluten sensitivity, a strict gluten-free diet might be therapeutically indicated. A prospective study on incident ALS cases with testing of the whole spectrum of gluten sensitivity–related antibodies and the possible effect of a gluten-free diet in patients with seropositive test results is ongoing at our center. Until more information on this association is gathered, we recommend a gluten-free diet only for patients with ALS who have a clear diagnosis of CD or NCGS. In any such instance, the diet should be closely monitored by an experienced dietician to avoid weight loss that might be deleterious for patients with ALS.


