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Increased Prevalence of Transglutaminase 6 Antibodies in Sera From Schizophrenia Patients

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Gluten can cause extraintestinal manifestations with or without gastrointestinal symptoms and elevated antitissue transglutaminase 2 (tTG2) autoantibodies. Organ-specific gluten reaction involves immune response toward other transglutaminase (TG) isoforms including tTG3 (expressed in the skin, leading to dermatitis herpetiformis) and tTG6 (expressed in the brain, causing gluten ataxia). This analysis focuses on tTG6 antibodies, which have never been studied before in schizophrenia (SZ) and its relationships to tTG2 and to antigliadin antibodies. We previously showed an increased prevalence of tTG2 antibodies in gluten sensitive SZ patients compared with healthy controls (HC) that was not paralleled by an increased prevalence of antiendomysial antibody. To elucidate this discrepancy, we examined those tTG2 positive SZ patients for the presence of tTG6 antibody. We also searched for tTG6 antibodies in our sample of antigliadin (AGA) positive and AGA and tTG2 negative SZ patients. Seventy-four tTG2 positive SZ patients were compared with 148 age and gender-matched HC. Of the 74 tTG2 positive SZ patients, 16 were positive for tTG6 IgA for a prevalence of 22%. Only 4 HC were positive for tTG6 IgA for a prevalence of 2.7%. Among the AGA positive SZ patients, the prevalence of tTG6 IgA was 21.3% while 13.1% of the AGA and tTG2 negative SZ patients were positive for tTG6 IgA. The HC had a prevalence of 6%. Our results indicate a higher prevalence of tTG6 antibodies in SZ that may represent a biomarker useful to identify SZ patients who would benefit from a gluten-free diet.

Key words: celiac disease/gluten sensitivity/ transglutaminase 6/gluten-free diet/schizophrenia

Introduction

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten-containing grains in genetically susceptible individuals. Tissue Transglutaminase 2 (TG 2) is the primary autoantigen of CD and antitissue transglutaminase 2 (tTG2) antibody is used as a serological marker for CD.

Gluten can also cause extraintestinal disease manifestations like the skin disorder dermatitis herpetiformis and a variety of neurological conditions, the most prevalent being gluten ataxia and gluten neuropathy.[1] These conditions can present with or without small intestinal symptoms and anti-tTG2 autoantibodies.[2,3] Dermatitis herpetiformis and gluten ataxia are, however, characterized by an immune response directed toward other tTG isoforms. Dermatitis herpetiformis patients have antibody populations primarily recognizing tTG3 (also known as epidermal transglutaminase) while gluten ataxia patients produce antibodies toward the more recently identified isoform tTG6,[4,5] a transglutaminase primarily expressed in the brain.[6] Antibodies rarely display cross-reactivity with different tTG isoforms suggesting that independent events may be involved in their development.[5] The isoforms tTG3 and tTG6 are now considered to be the main autoantigens in dermatitis herpetiformis and gluten ataxia, respectively, and these antibody populations should prove useful for the diagnosis of these diseases. Recent prevalence studies have shown that gluten sensitivity is increased in schizophrenia (SZ).[7,8] In our previously published report,[7] we found an increased prevalence of tTG positive SZ subjects compared with healthy controls (HC) (5.4% vs 0.8%, respectively), but a very low number of SZ patients were positive for antiendomysial antibody (EMA) (0.3%). Antibodies to endomysial tissue primarily target the tTG2 enzyme, and a discrepancy between EMA and tTG tests is usually detected in specific autoimmune diseases such as type-1 diabetes, autoimmune hepatitis, and autoimmune thyroid conditions revealing the existence of extraintestinal source of tTG.[9] We clarify this

discrepancy by assessing the prevalence of antibodies against tTG6, among those tTG positive SZ patients. We hypothesize that the discrepancy between EMA and tTG tests could be explained by an increased prevalence of antibodies against tTG6. We also assess the prevalence of tTG6 IgA antibodies in our antigliadin (AGA) positive subjects as well as in SZ patients negative for AGA and tTG antibodies. We hypothesize that like in gluten ataxia, SZ patients positive for AGA antibodies will show an increased prevalence of tTG6 antibodies compared with SZ patients negative for AGA antibodies and controls.

Methods

All of the tTG2 positive subjects from our previous investigation on the prevalence of CD and gluten sensitive (GS) involving SZ patients from the original Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study[7] were screened at the University of Maryland Center for Celiac Research for the presence of human neuronal tissue transglutaminase 6 (tTG6) antibodies IgA and IgG by enzyme-linked immunosorbent assay or ELISA using kits from Zedira GmbH (Darmstadt, Germany). The 74 tTG2 positive CATIE subjects and their matched controls used the original cutoff scores for the IgA antibodies recommended by the manufacturer: >21 high positive; 12–21 equivocal, and <12 negative. The cutoff scores for the IgG antibodies were: >38 high positive; 24–38 moderate positive, and <24 negative. The 249 AGA CATIE subjects and controls used the updated cutoff scores for the IgA antibodies that were recommended by the manufacture: >41 high positive, 26–41 equivocal, and <26 negative.

Each of the 74 tTG2 positive CATIE subjects were age and gender-matched with 2 HC drawn from the samples reported by Catassi et al.[10] We also screened for the presence of tTG6 IgA antibodies in a randomly selected 50% sample from the AGA positive SZ patients ($n = 160$) and 10% sample from the AGA and TG2 negative SZ patients ($n = 107$). We also calculated percentages of positive, equivocal, and negative tTG6 antibodies in the combined AGA positive and negative samples. HC ($n = 498$) were drawn from the same Catassi et al[10] sample. The χ^2 test for comparison of populations was applied to assess for statistical difference in the frequency of tTG6 antibodies.

Results

Of the 74 tTG2 positive CATIE samples, 16 were positive for tTG6 IgA antibodies for a prevalence of 21.6%, ie, about 10 times as high as the controls ($\chi^2 = 66.9$, $df = 1$, $P < .001$). Only 1 SZ subject and 2 of the controls were positive for the IgG antibodies. Of the 148 age and gender-matched controls, only 4 were positive for tTG6 IgA antibodies for a prevalence of 2.7%. Table 1 shows the demographic and clinical characteristics of the AGA positive and negative SZ samples. Table 2 shows the relation of antibodies to gliadin with antibodies to tTG6. Among the AGA positive SZ patients, the prevalence of tTG6 antibodies was 21.3% while 13.1% of the AGA and tTG2 negative SZ patients were positive for tTG6 antibodies. The difference between these 2 SZ groups was statistically significant ($\chi^2 = 7.71$, $df = 2$, $P < .05$). The 489 HC matched to the AGA positive and AGA plus tTG2 negative showed a 6% prevalence of tTG6 antibodies. The difference between the combined AGA SZ samples and controls was highly statistically significant ($\chi^2 = 96.43$, $df = 2$, $P < .001$) (see table 2). The prevalence for the entire CATIE sample was estimated using the inverse of the sampling probabilities (table 2: 15.0%).

We explored whether there might be diagnostic, demographic, or clinical differences between those in the tTG6 positive group vs those in the negative group. Given the small numbers and the weak power to detect differences, there were no statistically significant differences in gender, age, diagnosis, race/ethnicity, or Positive and Negative Syndrome Scale scores (table 1).

Discussion

The relevant finding of this study is the increased prevalence of tTG6 antibodies in SZ patients sera compared with HC.

In our previous report,[7] we showed an increased prevalence of AGA and tTG antibodies in SZ subjects compared with normal controls. The presence of AGA antibodies indicated that gliadin had been processed by the small intestinal epithelium, and a response was mounted by the immune system. We reported that a smaller percentage (5.4%) of SZ patients were tTG positive. We now show for the first time that a substantial subgroup of tTG positive patients is positive for antibodies against a tissue transglutaminase (tTG6) mainly expressed in the brain.[6] Our finding helps also to explain the discrepancy between a low percentage (0.3%) of EMA positive SZ patients vs tTG positive patients in the CATIE sample previously examined. Antibodies to endomysial tissue primarily target the tTG2 enzyme, and a discrepancy between EMA and tTG tests is usually detected in specific autoimmune diseases such as type-1 diabetes, autoimmune hepatitis, and autoimmune thyroid conditions revealing the existence of extraintestinal source of tTG.[9] Though we have not biopsied the intestine of the tTG6 positive subjects, our results closely resemble those of patients with gluten ataxia where gluten ataxia patients with enteropathy were EMA positive in 75% of the cases while those without intestinal involvement were all EMA negative.[5] Differently from patients with gluten ataxia where anti-tTG6 IgG antibodies were present in 90% of the cases and IgA absent in 21% of the

Table 1. Characteristics of AGA Positive and Negative Random SZ Samples From CATIE by tTG6 Levels

	AGA IgA Negative				AGA IgA Positive			
TTG6	Negative	Equivocal	Positive	Total	Negative	Equivocal	Positive	Total
Males	62	14	11	87	60	31	30	171
%	71.3	16.1	12.6	100	49.6	25.6	24.8	100
Females	13	4	3	20	25	10	4	39
%	65.0	20.0	15.0	100	64.1	25.6	10.3	100
Caucasian	51	10	7	68	45	20	21	86
%	75.0	14.7	10.3	100	52.3	23.3	24.4	100
Black/African American	20	7	5	32	35	20	13	68
%	62.5	21.9	15.6	100	51.5	29.4	19.1	100
Other	4	1	2	7	5	1	0	6
%	57.1	14.3	28.6	100	83.3	16.7	0.0	100
Schizophrenia[a]	70	18	13	101	81	41	30	152
Schizophreniform disorder	1	0	0	1	0	0	0	0
Schizoaffective disorder	4	0	1	5	4	0	4	8
Age at interview								
Mean years	40.6	44.0	36.9	40.7	41.7	42.5	44.3	42.4
SE	1.24	2.62	2.64	1.04	1.16	1.39	1.99	0.827
Total PANSS								
Mean score	74.5	77.2	84.1	76.2	78.99	74.3	74.8	76.894
SE	2.03	4.76	4.55	1.7	1.83	2.46	2.65	1.2915
PANSS positive								
Mean score	18.3	17.3	20.8	18.477	19.2	17.8	18.4	18.65
SE	0.63	1.17	1.43	0.52	0.57	0.92	0.88	0.427
PANSS negative								
Mean score	19.1	22.8	20.5	18.5	20.9	20.0	21.0	18.65
SE	0.73	1.82	1.46	0.52	0.74	0.90	1.02	0.427
PANSS psychopathology								
Mean score	37.0	37.0	42.9	37.8	38.9	36.6	35.4	37.55
SE	1.03	2.74	2.44	0.921	1.01	1.25	1.61	0.72

Note: AGA, antigliadin; SZ, schizophrenia; CATIE, Clinical Antipsychotic Trials of Intervention Effectiveness; PANSS, Positive and Negative Syndrome Scale.
[a]Primary diagnosis at CATIE screening.

cases, we found that only 1 of our SZ patients had anti-TG6 IgG antibodies. This finding is similar to that we have previously reported with AGA antibodies.[7]

The study findings agree also with our hypothesis that like in gluten ataxia we would find an increased prevalence of tTG6 antibodies in the AGA positive SZ sample compared with normal controls. As tTG6 is primarily expressed in the brain, the serum tTG6 IgA antibodies represent a marker of neuroinflammation. The most logical interpretation of our findings is that in GS SZ patients, gluten peptides (either directly or through activation of macrophages/dendritic cells) may set up an innate immune response in the brain similar to that described in the gut mucosa,[11] causing exposure of tTG6 from neuronal cells. Access of these gluten peptides and/or activated immune cells to the brain may be facilitated by a breach of the blood brain barrier. Evidence from the literature supports the notion that a subgroup of SZ patients shows increased expression of inflammatory markers including haptoglobin-2 chains α and β.[12] Interestingly, a tight junction modulator like zonulin whose release is triggered by specific gluten peptides[13] in the small intestine and whose receptor has been demonstrated in the human brain[14] has been identified as the precursor for haptoglobin-2.[15] Overexpression of zonulin (aka haptoglobin-2) could be involved in the blood-brain barrier disruption similarly to the role that zonulin plays in increasing intestinal permeability. This hypothesis is supported by the observation that zonulin analogues can modulate the blood brain barrier by increasing its permability to molecular weight markers and chemotherapeutic agents.[16]

The breaching of the blood brain barrier may also facilitate the passage of primed $CD4^+$ T cells that mounts an immunologic response to tTG6 once this enzyme has been exposed to the $CD4^+$ T cells as a result of neuroinflammatory events described above. Intrathecal origin of anti-tTG IgA and IgG class has been previously reported,[17] thus suggesting a production of these antibodies within the central nervous system (CNS). $CD4^+$ T memory cells from the gut and skin have also been previously identified in cerebro-spinal fluid (CSF).[18] Inflammatory events in the brain of SZ patients who are GS and centered around the brain vasculature is conceivable also on the basis of pathological findings in postmortem brain of gluten ataxia patients where perivascular cuffings of

Table 2. Relationship of Antibodies to Gliadin With Antibodies to tTG6 CATIE Samples and Healthy Controls

	AGA Antibodies in CATIE; Frequencies and Percentages				
Antibodies to tTG6	Negative	Positive	Combined Sample[a]	CATIE Total[b]	Healthy Controls
Negative	75 (70.1)	85 (53.1)	160 (59.9)	920 (66.2)	477 (89.3)
Equivocal	18 (16.8)	41 (25.6)	59 (22.1)	262 (18.8)	25 (4.7)
Positive	14 (13.1)	34 (21.3)	48 (21.3)	208 (15.0)	32 (6.0)
Total	107 (100.0)	160 (100.0)	267 (100.0)	1390 (100.0)	534 (100.0)

Note: AGA, antigliadin; SZ, schizophrenia; CATIE, Clinical Antipsychotic Trials of Intervention Effectiveness.
[a]Combined sample indicates the sum of AGA negative and positive SZ patients.
[b]Estimated using inverse of sampling probabilities.

immune complexes containing tTG6 and lymphocytes have been observed.[5]

Our data also show that AGA/tTG2 negative SZ patients have a prevalence of tTG6 antibodies more than double of the HC group (13.1% vs 6%, respectively). This finding appears to be in agreement with that of a previous report in non-SZ GS subjects that showed that 13 of 26 GS subjects were AGA and tTG negative.[11] It is then conceivable that our AGA/tTG2 negative SZ patients are also GS.

A limitation of the study is the lack of CSF titer for tTG6. It is important that CSF be investigated when serum derived CNS antibodies are studied. Another limitation is the lack of intestinal biopsy to assess the presence of enteropathy in the AGA positive and tTG2 positive SZ patients in order to compare our findings to those obtained in patients with gluten ataxia. A final limitation is in the observational nature of our study that does not allow conclusions on the causality of tTG6 antibodies in relation to SZ.

Nonetheless, we believe that the clinical knowledge about the presence of immunologic markers more directly related to the CNS in SZ patients with CD or gluten sensitivity may have implications for the treatment of these subjects given that gluten-free diet can potentially contribute to the improvement of their symptoms. In studies dating to the 1960s and 1970s, clinical trials of a gluten-free diet sometimes reported a high percentage of responders; later small trials in the 1980s did not always replicate these findings.[19] The ability to screen for CD and gluten sensitivity was not well developed until the 1990s, and all of these trials failed to screen patients for CD or gluten sensitivity, so that a high percentage of participants in these studies were unlikely to benefit from the intervention. Therefore, the identification of a potential biomarker of gluten sensitivity and neuroinflammation in SZ patients may provide the rationale for patients' stratification in order to specifically target those patients that can benefit from the implementation of a gluten free diet. To support this hypothesis, a gluten-free diet trial in SZ patients stratified by the presence of AGA, and tTG6 antibodies are necessary.

The results of the trial could finally clarify if gluten sensitivity contributes to the etiologic heterogeneity of SZ as a growing literature suggests that immune mechanisms are responsible for SZ or some proportion of it.[20]

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