Schizophrenia Bulletin vol. 38 no. 3 pp. 514–518, 2012
doi:10.1093/schbul/sbq111
Advance Access publication on September 30, 2010

A Study of Circulating Gliadin Antibodies in Schizophrenia Among a Chinese Population

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The present work measured circulating antibodies against native gliadins, deamidated gliadin–derived epitopes, and transglutaminase 2 (TGM2) in 473 patients with schizophrenia and 478 control subjects among a Chinese population. The results showed that 27.1% of patients with schizophrenia were positive for the IgA antibody against native gliadins compared with 17.8% of control subjects (χ^2 = 11.52, *P* = .0007, OR = 1.72, 95% CI 1.25–2.35), although this significant difference appeared to be due mainly to low IgA gliadin antibody levels in female controls. A total of 27.6% of female patients were positive for IgA gliadin antibodies compared with 13.9% of female controls (χ^2 = 10.46, *P* = .0012, OR = 2.36, 95% CI 1.39–4.01), and 26.4% of male patients were positive for IgA antibodies compared with 19.8% of male controls (χ^2 = 3.26, *P* = .071, OR = 1.46, 95% CI 0.97–2.19). Of 128 patients who were positive for the IgA antibody against native gliadins, 8 were positive for the IgA antibody against deamidated gliadin epitopes and 1 was positive for IgA anti-TGM2 antibody. However, quantitative analysis demonstrated that the mean levels of IgA antibodies against deamidated gliadin epitopes and TGM2 were significantly lower in patients with schizophrenia than the control subjects (*P* < .001 and *P* = .008, respectively). The prevalence of IgG antibodies against native gliadins was not significantly different between the patient group and the control group (χ^2 = 2.25, *P* = .134, OR = 1.32, 95% CI 0.92–1.88). This study suggests that specific gliadin-derived epitopes may be involved in schizophrenia.

Key words: gliadin antibody/TGM2 antibody/wheat gluten/schizophrenia

Introduction

Gluten is a protein from wheat, rye, and barley. Wheat gluten comprises two main components: gliadin and glutenin. Gliadin is thought as the principal toxic component for coeliac disease. Biochemical studies revealed that gliadin proteins contain some peptide sequences strongly resistant to digestive enzymes in the gut.[1] The undigested gliadin fragments will enter the body when gut permeability is increased by stress, infection, toxic chemicals, and other physical factors. The immune system will then be triggered to produce antibodies against the gliadin fragments. Antibodies can also be generated against tissue transglutaminase, also named transglutaminase 2 (TGM2) that has a high affinity for gliadins and can then modify gliadin fragments by deamidation and transamidation. While the mechanism of anti-TGM2 antibody production has not been proven, it is proposed that transamidation can result in cross-linking between gliadin fragments and TGM2, leading to creation of autoantigens for secretion of TGM2 antibody.[2,3] The anti-TGM2 antibody is a hallmark of coeliac disease.[4]

Apart from coeliac disease, many other conditions have been suggested to be associated with gluten ingestion, so-called gluten-associated diseases, including type-1 diabetes, schizophrenia, autism, and a wide range of neurological diseases.[5] Graff and Handford[6] were the first to report on high risk of schizophrenia in patients with coeliac disease. Clinical and immunological studies gave further evidence in support of the gluten hypothesis of schizophrenia.[7–17] A question is why gliadin-derived epitopes are involved in different diseases? A couple of recent studies suggest that gluten-derived epitopes for schizophrenia may be different from those for coeliac disease.[17,18] Samaroo et al[18] have carried out a pilot study

Table 1. The Prevalence of IgA Antibodies Against Native Gliadins in Patients With Schizophrenia and Healthy Subjects

Sex	Subject	Plasma IgA		χ^2	*P*	OR	95% CI
		Negative *N* (%)	Positive *N* (%)				
Male	Patients	167 (73.6)	60 (26.4)	3.26	.071	1.46	0.97–2.19
	Controls	243 (80.2)	60 (19.8)				
Female	Patients	178 (72.4)	68 (27.6)	10.46	.0012	2.36	1.39–4.01
	Controls	136 (86.1)	22 (13.9)				
Both	Patients	345 (72.9)	128 (27.1)	11.52	.0007	1.72	1.25–2.35
	Controls	379(82.2)	82 (17.8)				

in 17 schizophrenia patients who were positive for the antibodies against native gliadins (a complex mixture of up to 100 homologous proteins) and revealed that these 17 patients were all negative for IgA antibodies against a deamidated form of gluten-derived epitopes, which was developed to specifically detect circulating antigliadin antibodies in coeliac disease.[19] Dickerson et al[17] also found that while the prevalence of antigliadin antibodies was significantly higher in patients with schizophrenia than the control subjects, there was no significant difference in the prevalence of antibodies against deamidated gliadin-derived epitopes between the patient group and the control group. However, all the studies reported to date were performed in American populations (Caucasian and African American), although we have published a meeting abstract with a preliminary result from a study in a Chinese population.[15] The present study, therefore, reported in more detail on whether gluten-derived epitopes for schizophrenia are different from those for coeliac disease in a Han Chinese population.

Methods

This study was approved by an Ethics Committee of Jilin University, Changchun, China, with all subjects giving written informed consent. A total of 473 patients with schizophrenia, aged 38.9 ± 12.5 years, were recruited, and they were diagnosed as having schizophrenia independently by two consultant psychiatrists using the *Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems* (ICD-10).[20] These patients had been ill for at least a year at the time of sampling, and they all were on neuroleptic medication. Inclusion into the study was restricted to those with a diagnosis of schizophrenia only. We also recruited 478 healthy subjects, aged 39.4 ±13.0 years, as controls from local communities. Neither the patients with schizophrenia nor the control subjects had a history of coeliac disease, type-1 diabetes, and other clinically diagnosed autoimmune diseases. Those who were on wheat-free diets were excluded.

A 5-ml blood sample was drawn from each subject and plasma was separated by centrifugation for antibody assay. The enzyme-linked immunosorbent assay (ELISA) test kits for IgA antibodies to native gliadins, deamidated gliadin–derived epitopes (gliadin-related GAF-3X peptides), and TGM2 were purchased from EUROIMMUN (Beijing, China). Plasma samples were initially measured with native gliadin ELISA antibody kit. The subjects who were positive for native gliadin antibodies had further tests with anti-GAF-3X and anti-TGM2 ELISA antibody test kits. The antibody assay was performed as per the supplied instructions, and plasma antibody levels were expressed as relative units (RU/ml). As recommended by the manufacturer for the subjects aged over 4 years, <25 RU/ml and <20 RU/ml were used as the cutoff values of negative levels for anti-gliadin and anti-TGM2 antibodies, respectively. While most studies reported to date have mainly focused on detecting IgA antibodies in schizophrenia,[13,15,16] we also tested IgG antibodies to native gliadins with the ELISA test kits from EUROIMMUN in this study. The cutoff value of negative levels for IgG gliadin antibodies was the same as for IgA gliadin antibodies.

The software SPSS for Windows 14.0 was applied to perform the chi-square (χ^2) test in order to test the difference in the prevalence of gliadin antibodies between the patient group and the control group, with calculation of OR and 95% CI. To compare the difference in quantitative levels of gliadin antibodies between the two groups, the individual concentrations of antibodies were converted into logarithmic values and then analyzed by the Student's *t* test with SPSS for Windows 14.0. All the antibody concentrations of <1 RU/ml were treated as a logarithmic value of "0." Cohen's standard difference (*d*) and 95% CI were calculated using the software SPSS SamplePower 2.0.

Results

The prevalence of IgA native gliadin antibodies was significantly higher in patients with schizophrenia than in control subjects (χ^2 = 11.52, *P* = .0007, OR = 1.72, 95% CI 1.25–2.35). As shown in table 1, 27.1% of patients were positive for IgA gliadin antibodies compared with 17.8% of control subjects. This significant difference appeared to be due mainly to a low IgA gliadin antibody level in female controls. A total of 27.6% of female patients were positive for IgA gliadin antibodies

Table 2. Quantitative Levels of IgA Antibodies Against Native Gliadins in Patients With Schizophrenia and Healthy Subjects

Sex	Patients (*N*)	Controls (*N*)	*t*	*df*	*P*	*d*[a]	95% CI
Male	1.061 ± 0.542[b] (227)	0. 975 ± 0.528 (303)	1.848	528	.065	0.161	−0.011 to 0.333
Female	1.071 ± 0. 516 (246)	0. 907 ± 0. 455 (158)	3.268	402	.001	0.333	0.132–0.533
Both	1.066 ± 0. 528 (473)	0.951 ± 0. 505 (461)	3.399	932	.001	0.223	0.094–0.351

[a]The *d* value represents effect size, which was calculated with SPSS SamplePower 2.0.
[b]The value is logarithmic in IgA RU/ml (Mean ± SD).

compared with 13.9% of female controls ($\chi^2 = 10.46$, $P = .0012$, OR = 2.36, 95% CI 1.39–4.01), whereas 26.4% of male patients were positive for IgA gliadin antibodies compared with 19.8% of male controls ($\chi^2 = 3.26$, $P = .071$, OR = 1.46, 95% CI 0.97–2.19). Quantitative analysis further confirmed that the mean levels of the IgA antibody against native gliadins were significantly higher in patients with schizophrenia than the control subjects ($t = 3.399$, $df = 932$, $P = .001$, $d = 0.223$, 95% CI 0.094–0.351) and that female patients mainly contributed to the increased antibody levels (table 2).

Of those who were positive for the IgA antibody against native gliadins, 6.25% of patients (8/128) were positive for the IgA antibody against deamidated gliadin epitopes compared with 12.2% of control subjects (10/82) and 0.8% of patients (1/128) were positive for the IgA antibody against TGM2 compared with 1.2% of control subjects (1/82). Although the prevalence of IgA antibodies against deamidated gliadin epitopes and TGM2 did not show a significant difference between the patient group and the control group, as shown in table 3, the mean levels of IgA antibodies against them were significantly lower in patients with schizophrenia than in the control subjects ($t = -3.871$, $df = 208$, $P < .001$ for deamidated gliadin epitopes and $t = -2.692$, $df = 208$, $P = 0.008$ for TGM2, respectively).

The prevalence of plasma IgG antibodies against native gliadins did not show significant differences between the patient group and the control group (table 4). Only 17.9% of patients with schizophrenia were positive for IgG gliadin antibodies compared with 14.2% of control subjects ($\chi^2 = 2.25$, $P = .134$, OR = 1.32, 95% CI 0.92–1.88). Quantitative analysis also failed to show a difference in IgG gliadin antibody levels between the patient group and the control group (table 5).

Discussion

The present study provides further evidence in support of the hypothesis that gluten consumption may be an environmental factor for a subgroup of schizophrenia. It is worth noting that the prevalence of circulating IgA gliadin antibodies was significantly higher in female patients but only marginally higher in male patients than control subjects (table 1). Cascella et al[16] also showed a gender difference in the prevalence of plasma IgA gliadin antibodies, in which the IgA antibody level was slightly higher in female patients than in male patients, although this difference did not achieve a statistical significance. Possibly, the gender differences in gliadin antibody secretion reflect etiological heterogeneity of schizophrenia, which may lead to clinical heterogeneity of the illness. Gender-specific clinical presentation has been documented for many years. Men with schizophrenia exhibit an early age of onset, poor premorbid functioning, severe negative symptoms, differential neuron cognitive functioning, reduced emotion perception, and a poor response to neuroleptic medication as well as differential structural and functional brain abnormalities.[21–25] In contrast to men, the progress of schizophrenia in women shows more positive and affective symptoms, lower levels of disability, better premorbid functioning, and superior integration into the community.[25,26] It will be important to investigate whether the gluten-derived epitopes are a sex-specific environmental factor for schizophrenia.

In addition, the baseline of circulating antigliadin antibody levels appears to differ between the Chinese

Table 3. Quantitative Levels of IgA Antibodies Against TGM2 and Deamidated Gliadin Epitopes in the Subjects Who Were Positive for the IgA Antibody to Native Gliadins

Antigen	Patients (*N*)	Controls (*N*)	*t*	*df*	*P*	*d*[a]	95% CI
TGM2	0.267 ± 0.323[b] (128)	0.398 ± 0.375 (82)	−2.692	208	.008	−0.381	−0.659 to −0.102
Deamidated epitopes	0.776 ± 0.491 (128)	1.030 ± 0.420 (82)	−3.871	208	<.001	−0.547	−0.825 to −0.268

[a]The *d* value represents effect size, which was calculated with SPSS SamplePower 2.0.
[b]The value is logarithmic in IgA RU/ml (Mean ± SD).

Table 4. The Prevalence of IgG Antibodies Against Native Gliadins in Patients With Schizophrenia and Healthy Subjects

Sex	Subjects	IgG in Plasma		χ^2	*P*	OR	95% CI
		Negative *N* (%)	Positive *N* (%)				
Male	Patients	143 (82.7)	30 (17.3)	0.48	.487	1.19	0.73–1.95
	Controls	295 (85.0)	52 (15.0)				
Female	Patients	201 (81.7)	45 (18.3)	2.33	.127	1.61	0.87–2.98
	Controls	115 (88.5)	16 (12.3)				
Both	Patients	344 (82.1)	75(17.9)	2.25	.134	1.32	0.92–1.88
	Controls	410(85.8)	68 (14.2)				

population and the other populations.[7,16] This may be the major reason why the IgA gliadin antibody shows a smaller effect size in the Chinese population, although a significant difference in the antibody levels was observed between the patient group and the control group (tables 1 and 2). Dickerson et al[17] reported on an increase in IgG gliadin antibody levels in patients with schizophrenia in the American population, but we failed to replicate the IgG finding in the Chinese population (tables 4 and 5). Ethnic background may be a confounding effect on the prevalence of antigliadin antibodies.

The GAF-3X ELISA antibody kit has been developed to specifically detect the antibodies against deamidated gliadin epitopes, and approximately 90% of patients with coeliac disease were positive.[19] In this study, we found that only 6.25% of patients who were positive for IgA native gliadin antibodies were positive for the IgA antibody against deamidated gliadin epitopes; we also found that <1% patients who were positive for IgA native gliadin antibodies were positive for the IgA antibody against TGM2. Quantitative analysis showed that the mean levels of IgA antibodies against deamidated gliadin epitopes and TGM2 were significantly lower in the patient group than in the control group (table 3). These results suggest that schizophrenia may involve a distinct immunological mechanism by which gliadin-derived epitopes trigger production of their specific antibodies in comparison with coeliac disease. The results from other studies also support this possibility, although Cascella et al[16] found that 5.4% of schizophrenia patients had moderate to high levels of IgA TGM2 antibodies compared with 0.8% of the healthy subjects; however, these authors did not compare the difference in quantitative levels of plasma anti-TGM2 antibodies between the patient group and the control group. Dickerson et al[17] have recently performed quantitative analysis of IgA and IgG antibodies against TGM2 in an American population and found that only anti-TGM2 IgG antibody levels were significantly higher in patients with recent-onset psychosis than those with multiepisode schizophrenia and nonpsychiatric controls. Moreover, increased levels of antibodies against deamidated gliadin–derived epitopes and TGM2 in the healthy population also suggest that the Chinese population may be more sensitive to wheat gluten than other populations. Large-scale screening of antigliadin antibodies in the Chinese population may be very useful to confirm the difference in gluten sensitivity between subpopulations.

Gliadin is a superfamily with hundreds of homologous sequences, including α-/β-gliadins, γ-gliadins, and ω-gliadins. The most toxic fragment of gliadin for coeliac disease is a 33-mer peptide derived from α2-gliadin, which contains several overlapping human leukocyte antigen (HLA)-DQ2 restricted T-cell epitopes.[1] The HLA alleles encoding HLA-DQ2 molecules that mainly present deamidated gluten–derived antigens, such as HLA-DQ2.5, confer a genetic risk of coeliac disease and >90% of patients with coeliac disease carry the HLA-DQ2 alleles.[4] A genome-wide association study has recently confirmed that the HLA-DQA*0501 and HLA-DQB*0201 alleles, which encode HLA-DQ2.5 molecules, are negatively associated with schizophrenia.[27] It is unlikely that HLA-DQ2.5 molecules mediate the generation of gluten antibodies in schizophrenia. While coeliac disease and schizophrenia immunologically overlap one another, these two conditions may be developed through different immunogenetic pathways.

Table 5. Quantitative Levels of IgG Antibodies Against Native Gliadins in Patients With Schizophrenia and Healthy Subjects

Sex	Patients (*N*)	Controls (*N*)	*t*	*df*	*P*	*d*[a]	95% CI
Male	0.914 ± 0. 571[b] (173)	0.826 ± 0.558 (347)	1.694	518	.091	0.156	−0.026 to −0.339
Female	0.853 ± 0. 598 (246)	0.864 ± 0.538 (131)	−0.178	375	.858	−0.019	−0.232 to 0.193
Both	0.878 ± 0. 587 (419)	0.836 ± 0.552 (478)	1.106	895	.269	0.074	−0.057 to 0.205

[a]The *d* value represents effect size, which was calculated with SPSS SamplePower 2.0.
[b]The value is logarithmic in IgG RU/ml (Mean ± SD).

Further investigation is needed to identify which gluten-derived epitope is specifically involved in schizophrenia by acting on a certain HLA-DQ variant.

Funding

National Natural Science Foundation of China (30700257); 985 Project Post-graduate Innovation Fund of Jilin University (20080222).

Acknowledgments

We thank the patients and healthy volunteers for their support and participation. We also thank Kaixuan Hospital, Changchun, China, for clinical interview with subjects and collection of blood samples.
The Authors have declared that there are no conflicts of interest in relation to the subject of this study.

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