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A study of anti-gliadin antibodies in first-episode patients with schizophrenia among a Chinese population

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ABSTRACT

A recent study suggested that digestion-resistant peptides derived from wheat gluten (mainly gliadin) could induce the secretion of anti-gliadin IgG antibodies in patients with schizophrenia. This research was then designed to replicate this initial finding in 134 drug-naïve patients with first-episode schizophrenia and 160 healthy controls. An enzyme-linked immunosorbent assay was developed in-house with 8 gliadin-derived peptide antigens to test anti-gliadin IgG antibodies in the circulation. The results showed that schizophrenia patients had significantly higher levels of plasma anti-AL2G2 IgG and anti-ABO3a IgG than healthy controls. Based on the specificity of 95%, anti-AL2G2 IgG assay had a sensitivity of 12.7% and anti-ABO3a IgG assay had a sensitivity of 17.2% for anti-ABO3a IgG assay. Increased levels of anti-AL2G2 and anti-ABC3a IgG antibodies were not correlated with total IgG levels in either the patient group or the control group. In conclusion, circulating IgG against AL2G2 and ABO3a may be useful biomarkers for identification of a gluten-sensitive subgroup of schizophrenia in the Chinese population although the present results are rather different from the work performed in a British population.

1. Introduction

Schizophrenia is a leading mental health problem with a lifetime prevalence of approximately 1%, and places an enormous burden on family, society and healthcare providers. It is characterized by abnormal social behavior with combination of positive, negative and cognitive symptoms. Etiologically, a genetic component is involved in developing the disease and its heritability has been estimated up to 80% (Cardno and Gottesman, 2000; Sullivan et al., 2003; International Schizophrenia Consortium et al., 2009). A genome-wide association (GWA) study has confirmed that 108 loci in the human genome are very likely to harbor a gene for schizophrenia, in which most genes are highly expressed not only in the brain but also in B-lymphocytes (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). The treatment of schizophrenia mainly relies on antipsychotic medication although antipsychotic drugs are not always efficacious and severe side effects are often observed (Ackenheil and Weber, 2004; Solanki et al., 2009). There is a need to identify a biomarker being used for the development of precision treatment of patients with schizophrenia.

In recent years, dysfunction of the immune system has been frequently reported (Perry et al., 1979; Abi-Dargham et al., 1998; Ezeoke et al., 2013; Khandaker et al., 2015; Severance et al., 2016, 2018); GWA studies have also revealed that the strongest association signal was identified in the human leukocyte antigen (HLA) locus (International Schizophrenia Consortium et al., 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Interestingly, epidemiologic studies suggested that schizophrenia was very likely to be associated with wheat consumption (Graff and Handford, 1961; Dohan, 1966; Jackson et al., 2012) and a large proportion of schizophrenia patients were found to carry anti-gliadin antibodies in their circulation (Reichelt and Landmark, 1995; Dickerson et al., 2010; Cascella et al., 2011; Jin et al., 2012; Okusaga et al., 2013; Lachance and Mckenzie, 2014; McLean et al., 2017; Čiháková et al., 2018). Because most studies performed with anti-gliadin antibody test have applied the enzyme-linked immunosorbent assay (ELISA) made from the mixture of native gliadin molecules, a recent study developed an in-house ELISA with digestion-resistant peptide fragments derived from native gliadins, and they found that the change of circulating anti-gliadin antibody levels was different from previous reports (McLean et al., 2017). In fact, gliadin molecules are rich in proline that is strongly resistant to digestive enzymes such as pepsin, trypsin and

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chymotrypsin in the gut. Therefore, the measurement of circulating antibodies for indigested gliadin-derived fragment may be more meaningful in immunological research into schizophrenia. However, the previous study conducted by McLean et al. (2017) detected plasma antibodies for gliadin-derived peptide antigens only in medicated patients. For this reason, the present study was designed to replicate the initial finding in drug-naïve patients with first-episode schizophrenia.

2. Materials and methods

2.1. Subjects

A total of 134 patients aged 36.3 ± 15.4 years (61 males and 73 females) were recruited between January and July 2017 by the Third People's Hospital of Jiangmen, Jiangmen, China. All patients were diagnosed as having first-episode schizophrenia by consultant psychiatrists using the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10), and they were all drug-naïve. Those patients whose diagnosis was changed after antipsychotic medication were not included in this study. Simultaneously, 160 healthy controls, aged 36.0 ± 12.8 years (77 males and 83 females) were recruited from local communities during the same period; eligible control subjects did not have history or current diagnosis of a mental health condition. The participants who had history of either an autoimmune condition or a malignant disease were excluded from this study. All subjects were of the Chinese Han origin and they all gave written informed consent to participate in this study. A 5 ml volume of blood samples was collected from each participant for separation of plasma and all blood samples were collected from patients just before they received antipsychotic medication. This study was approved by a local ethics committee and conformed to the provisions of the Declaration of Helsinki.

2.2. Detection of IgG antibodies against peptide antigens

An ELISA was developed in-house to detect plasma IgG antibodies against gliadin-derived fragments as reported in a recent study (McLean et al., 2017). In brief, these peptide antigens were synthesised by solid-phase chemistry with a purity of >95% and dissolved in 67% acetic acid to a concentration of 5 mg/ml as stock solution; the working solution of 20 µg/ml was made by dilution in coating buffer (0.1 M phosphate buffer containing 0.15 M NaCl and 10 mM EDTA, pH 7.2). Maleimide-activated 96-well plates (ThermoFisher Scientific, Shanghai, China) were coated with 100 µl of the working solution and incubated overnight at 4 °C (Hallford et al., 2016; Whelan et al., 2018). Antigen-coated plates were washed twice with 200 µl of wash buffer (phosphate-buffered saline, PBS containing 0.1% Tween-20) before plasma samples were added. All plasma samples including positive control (PC) diluted 1:100 in assay buffer (PBS containing 0.5% bovine serum albumin) and 50 µl of the sample was loaded into each sample well; 50 µl of assay buffer was added to each negative control (NC) well. After incubation at room temperature for 90 min, the plate was washed three times with wash buffer and 50 µl of peroxidase-conjugated goat anti-human IgG Fc (ab98624, Abcam, Guangzhou, China) diluted 1:50,000 in assay buffer was then added and incubated for 60 min at room temperature. The plate was washed three times with 200 µl of wash buffer before 50 µl of 3,3′,5,5′-tetramethylbenzidine (SB02, Life Technologies, Guangzhou, China) was added, and the plate was incubated in the dark for 20 min before 25 µl of the stop solution was added (SS04, Life Technologies). The optical density (OD) of each well was measured within 10 min with a plate reader at 450 nm with a reference wavelength of 620 nm. All samples were tested in duplicate and the specific binding ratio (SBR) was calculated based on previous reports (Hallford et al., 2016; Whelan et al., 2018):

$$SBR = (OD_{sample} - -OD_{NC})/(OD_{PC} - -OD_{NC})$$

2.3. Detection of total IgG levels in plasma

The Human IgG total ELISA Ready-SET-Go kit (Thermo eBioscience, Guangzhou, China) was used to test the total IgG antibodies in plasma by following manufacturer's instruction. In brief, the capture antibody (purified anti-human IgG monoclonal antibody) was diluted 250 times in coating buffer and added 100 µl to each sample well on 96-well plate. After incubation at 4 °C overnight, the plate was washed twice with Washing Buffer and 100 µl of standards and pre-diluted plasma samples (100,000-fold in Assay Buffer A) were then added to individual wells. After incubation for two hours and washing four times, 100 µl detection antibody (HRP-conjugated anti-human IgG monoclonal antibody, diluted 1:250 in Assay Buffer A) was added to each well that was then incubated for an hour. Color development was performed with 100 µl Substrate Solution added to each well and incubated at room temperature for 20 min before 100 µl of Stop Solution was added. OD was determined with a plate reader at 450 nm and the standard curve was used to calculate the total IgG levels in plasma.

2.4. Data analysis

Kolmogorov–Smirnov test showed a normal distribution only in plasma anti-AL2G2 IgG antibodies in both the patient group and the control group, so that Mann–Whitney *U test* was applied to examine the differences in plasma anti-gliadin IgG levels between the two groups. Spearman correlation was applied to analyze the correlation between plasma IgG against individual gliadin-derived antigens and total IgG antibodies. To reduce the type-I errors due to multiple testing, $p < 0.0063$ was considered to be statistically significant based on the Bonferroni correction. Receiver operating characteristic (ROC) curve analysis was applied to work out the area under the ROC curve (AUC) with calculation of a sensitivity against >95% specificity. A quality control sample (QC) that was pooled plasma from >50 individuals randomly selected from a healthy population, and the QC plasma sample was tested on every plate. The coefficients of variation (CV) were calculated based on 14 QC tests and used to access the reproducibility of this in-house ELISA.

3. Results

The in-house ELISA showed a good reproducibility based on the CV calculated from 14 tests with the QC sample (Table 1), in which the anti-ABO3a IgG assay showed the lowest CV (5.13%) and the anti-AAQ6A IgG assay showed the highest CV (19.26%).

Mann–Whitney *U* test showed that patients with schizophrenia had significantly higher levels of plasma anti-AL2G2 IgG ($Z = -3.35$, $p < 0.001$) and anti-ABO3a IgG ($Z = -2.89$, $p = 0.004$) than control subjects (Table 2). However, circulating levels of IgG antibodies for other 6 gliadin-derived antigens did not show a significant change in schizophrenia (Table 2). ROC curve analysis demonstrated that the anti-ABO3a IgG assay showed the best sensitivity of 17.2% against specificity of 95% (Table 3), while the anti-AL2G2 IgG assay showed the largest AUC of 0.61 (Table 3).

Table 1
The reproducibility of the in-house ELISA in all 8 IgG tests.

Antigen Mean ± SD (*n*)	CV (%)
AL1G1 3.06 ± 0.44 (14)	14.38
AL2G1 2.28 ± 0.38 (14)	16.67
AL2G2 1.13 ± 0.07 (14)	6.19
AAQ6A 2.28 ± 0.45 (14)	19.74
AAQ6B 0.92 ± 0.07 (14)	7.61
AAQ6C 1.01 ± 0.09 (14)	8.91
ABO3a 1.56 ± 0.08 (14)	5.13
ABO3b 1.34 ± 0.09 (14)	6.72

Table 2
Circulating levels of anti-gliadin IgG in schizophrenic cases and controls.

Antigen type	Patient group Mean ± SD	Control group Mean ± SD	Z[a]	p[b]
AL1G1	1.85 ± 2.10	2.60 ± 4.04	0.79	0.429
AL2G1	1.40 ± 0.78	1.47 ± 0.74	−1.06	0.290
AL2G2	1.00 ± 0.23	0.92 ± 0.21	3.35	<0.001
AAQ6A	1.47 ± 0.99	1.38 ± 0.71	0.003	0.997
AAQ6B	0.58 ± 0.23	0.53 ± 0.23	1.92	0.056
AAQ6C	0.69 ± 0.25	0.67 ± 0.23	0.59	0.553
ABO3a	1.29 ± 0.31	1.19 ± 0.27	2.89	0.004
ABO3b	0.99 ± 0.35	0.92 ± 0.33	1.63	0.104

[a] Mann–Whitney U test was used to test the differences in plasma anti-gliadin IgG levels between patients with first episode schizophrenia and healthy controls.
[b] Based on the Bonferroni correction, $p < 0.0063$ was considered to be statistically significant based on the Bonferroni correction.

Table 3
ROC curve analysis of plasma anti-gliadin IgG levels in schizophrenia.

Antigen	Sensitivity (%)[a]	AUC	SE[b]	95% CI
AL1G1	2.2	0.53	0.034	0.460–0.593
AL2G1	5.0	0.54	0.034	0.469–0.602
AL2G2	12.7	0.61	0.033	0.549–0.678
AAQ6A	5.0	0.50	0.034	0.433–0.567
AAQ6B	4.5	0.57	0.034	0.499–0.631
AAQ6C	6.7	0.52	0.034	0.453–0.587
ABO3a	17.2	0.60	0.033	0.532–0.663
ABO3b	7.5	0.56	0.034	0.489–0.621

[a] Specificity of 95%.
[b] Standard error.

Table 4
The correlation between the total IgG levels and individual anti-gliadin IgG levels in patients with schizophrenia.

antigen type	r	df	p[a]
AL1G1	−0.087	132	0.320
AL2G1	0.115	132	0.186
AL2G2	0.199	132	0.021
AAQ6A	0.050	132	0.565
AAQ6B	0.151	132	0.081
AAQ6C	0.121	132	0.163
ABO3a	0.149	132	0.085
ABO3b	0.143	132	0.099

[a] $p < 0.0063$ was considered to statistically significant based on the Bonferroni correction.

Table 5
The correlation between the total IgG levels and individual anti-gliadin IgG levels in control subjects.

antigen type	r	df	p[a]
AL1G1	0.084	158	0.292
AL2G1	0.176	158	0.026
AL2G2	−0.026	158	0.744
AAQ6A	0.118	158	0.136
AAQ6B	0.188	158	0.018
AAQ6C	0.042	158	0.601
ABO3a	0.087	158	0.276
ABO3b	−0.041	158	0.608

[a] $p < 0.0063$ was considered to statistically significant based on the Bonferroni correction.

There was no significant difference in total IgG levels between the patient group and the control group ($Z = 0.31$, $p = 0.756$) although the total IgG levels were slightly higher in patients with schizophrenia (2.71 ± 1.58 mg/ml) than control subjects (2.64 ± 1.40 mg/ml). Spearman correlation analysis showed no correlation between plasma IgG against individual gliadin-derived antigens and total IgG antibodies either in the patient group (Table 4) or in the control group (Table 5).

4. Discussion

Several lines of evidence have suggested that schizophrenia is very likely to be associated with wheat consumption (Graff and Handford, 1961; Dohan, 1966; Dohan and Grasberger, 1973; Samaroo et al., 2010; Jackson et al., 2012) and patients with schizophrenia may have abnormal immune responses to wheat gluten (Dickerson et al., 2010; Cascella et al., 2011; Jin et al., 2012; Okusaga et al., 2013; Lachance and Mckenzie, 2014; McLean et al., 2017; Čiháková et al., 2018). In this study, we applied an in-house ELISA that is different from that reported by McLean et al. (2017) to replicate their initial work in a Chinese population. While we failed to find that plasma anti-AAQ6C IgG levels were significantly increased in schizophrenia, circulating levels of anti-AL2G2 IgG and anti-ABO3a IgG were significantly higher in the patients with first-episode schizophrenia than healthy controls (Table 2). Because all patients were drug- naïve, our results are very likely to reflect a true relationship between wheat consumption and schizophrenia although this work has yet been replicated. It is worth noting that increased levels of anti-AL2G2 IgG and anti-ABC3a IgG were not correlated with the total IgG levels in the patient group (Table 4), suggesting that the alteration of anti-AL2G2 and anti-ABO3a IgG levels is likely to be specific for schizophrenia. Based on the present work, plasma anti-AL2G2 IgG and anti-ABO3a IgG may be useful biomarkers for a gluten-sensitive group of schizophrenia although this in-house ELISA is not sensitive enough for clinical diagnosis. In fact, schizophrenia is etiologically heterogeneous, and gluten-associated schizophrenia may represent a subgroup of the diseases. If the detection of anti-gluten IgG could identify 10–20% of schizophrenia patients who are sensitive to gluten intake, the test should be good enough for the development of precision medication. Furthermore, the gastrointestinal tract (GI) is the largest immune organ in the body (Severance et al., 2015). The immune response to indigestible wheat gluten-derived fragments in the gut may trigger GI inflammation that may induce neuroinflammation in the brain via the vague nerve system (Dinan et al., 2014).

There is a limitation of this study. It may be important to investigate the relationship between circulating anti-gliadin antibody levels and the response of schizophrenia patients to antipsychotic medication. Unfortunately, this study did not perform clinical follow-up to explore this relationship. Further investigation of this issue will be carried out in future work.

Conflict of interests

All authors declared that they had no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.12.161.

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