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Differential antibody responses to gliadin-derived indigestible peptides in patients with schizophrenia

RT McLean¹, P Wilson², D St Clair³, CJ Mustard¹ and J Wei¹

Gluten consumption has previously been implicated in the development of schizophrenia while an immunological link between gluten and schizophrenia was established by the detection of circulating antibodies against gliadin, a major component of wheat gluten. Several studies have reported an increase in circulating antibodies against native gliadin molecules that are unlikely to survive degradation in the digestive system. In this study, therefore, we measured plasma immunoglobulin G (IgG) and IgA antibodies against indigestible gliadin-derived peptide antigens using an in-house enzyme-linked immunosorbent assay (ELISA) among 169 patients with schizophrenia and 236 control subjects. We also examined the plasma levels of IgG and IgA antibodies against the mixture of native gliadins using commercially available ELISA kits. The results showed that patients with schizophrenia had the increased levels of plasma IgG against the γ-gliadin-derived fragment, namely AAQ6C, but decreased levels of plasma IgG against the α- and γ3-gliadin-derived antigens, as compared with control subjects. This study also demonstrated a uniform decrease in plasma IgA antibodies against gliadin-derived antigens. There was no significant difference in the levels of plasma antibodies against native gliadins between the patient group and the control group. Of eight gliadin-derived antigens tested, four showed a sensitivity of >20% against the specificity of ≥95% for detection of their corresponding antibodies in plasma. These four tests may thus have a potential to serve as biomarkers for the identification of schizophrenia subgroups that may need an alternative therapy or precision treatment. Further investigation with clinical trials should be carried out to explore this possibility.

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INTRODUCTION

Schizophrenia is a complex psychiatric disorder, demonstrating heterogeneity in clinical presentation with a combination of positive, negative and cognitive symptoms.¹ What causes schizophrenia remains unknown but alterations of neuronal communication are believed to underlie the pathophysiology of the illness.²–⁵ Owing to the diversity of clinical presentation, differences in treatment response and variable epidemiology, it is likely that multifactorial mechanisms contribute to a spectrum of schizophrenic illnesses.⁶–⁸

A role of gluten consumption in the development of schizophrenia was initially proposed based on the observation of a positive correlation between national wheat imports and hospital admissions for schizophrenia.⁵ Although the outcomes have been inconsistent, studies have attempted to examine the efficacy of gluten-free diets in the treatment of schizophrenia, demonstrating improvement of clinical scales and earlier recovery in some patients treated with gluten-free diets.¹⁰–¹⁵ Case studies in the literature have further demonstrated the induction of psychiatric and schizophrenia-like symptoms in response to gluten challenge and the resolution of these symptoms with gluten-free diets.¹⁴¹⁵

A mechanism by which gluten consumption may have a role in the development of schizophrenia has yet to be demonstrated. A number of immunological alterations have been found to be associated with schizophrenia, including an increase in pro-inflammatory cytokines and microglia activation.¹⁶–¹⁹ In addition, an increase in immunoglobulins (Ig) G and A classes against native gliadin, a major component of wheat gluten, was previously observed in a proportion of patients with schizophrenia.¹²–²⁰ The initiation of Ig production relies upon the recognition and presentation of antigens by the human leukocyte antigen class II (HLA-II) molecules. Genome-wide association studies revealed that the loci most strongly associated with schizophrenia resided in the HLA region.²⁶–²⁸

The epitopes recognized by anti-gliadin antibodies (AGAs) detected in schizophrenia may be different from those identified in the gluten-sensitive enteropathy coeliac disease (CD). Of schizophrenia patients who were positive for AGA IgA, only 3.8% were positive for IgA against CD-specific gliadin-derived epitopes, compared with 12.2% of control subjects.²²,²⁹ Furthermore, patients pre-selected for high AGA levels did not display high levels of CD-specific serological markers, such as plasma antibodies against tissue transglutaminase.³⁰ Previous studies suggested that the pathogenic gluten fragments for CD were mainly derived from α2-gliadin and γ5-gliadin, whereas immune reactivity to γ3-gliadin and its homologous sequence (NCBI accession AAQ6387) was associated with schizophrenia.³⁰–³²

To date, all the tests for circulating AGAs in schizophrenia have been developed with mixtures of full-length native gliadins consisting of ~300 amino acid residues. Such a test would detect antibodies against not only linear epitopes but also conformational epitopes that are unlikely to survive digestion in the gut. In this study, we measured plasma levels of IgG and IgA against indigestible peptide fragments derived from γ- and α-gliadins, which harbour HLA-II restricted epitopes, with an in-house enzyme-linked immunosorbent assay (ELISA) in individuals with...
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Table 1. Sequence information of peptide antigens used for the in-house ELISA

| NCBI accession (antigen) | Sequence | Position (aa) | Native molecule |
|--------------------------|----------|---------------|----------------|
| CAB76957 (ALG1G1)        | KCSFQQSQNPOAQGVSQVPQQLPQ | 205–226 | α1-Gliadin |
| CAB76964 (ALG2G1)        | CFFFRPPQQPQPOQYSPQPPQQPSQ | 88–111 | α2-Gliadin |
| CAB76964 (ALG2G2)        | KNVYIPPIYAPVVGFTGNYR | 270–290 | γ-Gliadin |
| AAQ6387 (AAQ6A)          | CHFIPQQPFFPQPQQPSQPPQQPSL | 59–72 | γ-Gliadin |
| AAQ6387 (AAQ6B)          | CHSIPQQPQEOQROGVQVQWLPQSL | 185–208 | γ-Gliadin |
| AAQ6387 (AAQ6C)          | HPKCSMRAPPASIVAGGYQYRD | 253–274 | γ-Gliadin |
| ABO37962 (ABO3a)         | KATTITANMQVDPQGQVWQPQPPRFRC | 13–38 | γ3-Gliadin |
| ABO37962 (ABO3b)         | KYVRPDCSTINAPASIVAGsisGH | 263–285 | γ3-Gliadin |

Abbreviation: ELISA, enzyme-linked immunosorbent assay. Peptide sequences were selected from in silico analysis of by PeptideCutter to determine indigestible fragments of >9 amino acid residues in length, which may have potential antigenicity.

schizophrenia and healthy controls. We also tested circulating AGAs in our case–control samples using commercially available ELISA kits.

MATERIALS AND METHODS

Subjects

A total of 405 archived plasma samples collected from patients with schizophrenia (n = 169, 132 males and 37 females, aged 42.0 ± 13.3 years) and control subjects (n = 236, 159 males and 77 females, aged 44.7 ± 12.5 years), were used to examine the levels of circulating antibodies against gliadin-derived peptide antigens. These samples were collected through the University of Aberdeen in the period between 2003 and 2008, and had been stored long-term at –80 °C without defrosting until they were aliquoted for antibody testing. All patients were diagnosed as having schizophrenia based on the DSM-IV criteria. Control subjects were recruited from a local population in Scotland and screened for psychiatric disorders, as described previously.25 No case samples were reported to have CD. Both case and control samples were collected in the same period and stored under the same conditions. Antipsychotic drugs used by schizophrenia patients at the time of sampling are listed in Supplementary Table 1, with 128 patients taking a single antipsychotic drug, 14 taking 2 antipsychotic drugs, and 29 taking 3 or more antipsychotic drugs. A total of 128 patients had been stored long-term at 80 °C without defrosting until they were used for this study. All case samples were tested on every 96-well plate, and expressed as a coefficient of variation (CV%) to represent the reproducibility of the in-house ELISA.

Antigen selection

Based on previous literature suggesting immune reactivity against the γ-gliadins in schizophrenia and α-gliadins in CD,30,32 sequences of interest were retrieved from the NCBI protein database (http://www.ncbi.nlm.nih.gov/protein). The sequences were analysed in silico to determine indigestible fragments using PeptideCutter software.33 The linear peptide antigens used in this study were selected on the basis of the presence of gliadin-derived peptide antigens. These samples were collected through the University of Aberdeen in the period between 2003 and 2008, and had been stored long-term at –80 °C without defrosting until they were aliquoted for antibody testing. All patients were diagnosed as having schizophrenia based on the DSM-IV criteria. Control subjects were recruited from a local population in Scotland and screened for psychiatric disorders, as described previously.25 No case samples were reported to have CD. Both case and control samples were collected in the same period and stored under the same conditions. Antipsychotic drugs used by schizophrenia patients at the time of sampling are listed in Supplementary Table 1, with 128 patients taking a single antipsychotic drug, 14 taking 2 antipsychotic drugs, and 29 taking 3 or more antipsychotic drugs. A total of 128 patients had been stored long-term at 80 °C without defrosting until they were used for this study. All case samples were tested on every 96-well plate, and expressed as a coefficient of variation (CV%) to represent the reproducibility of the in-house ELISA.

In-house ELISA for antibodies against gliadin-derived antigens

Each synthetic peptide was dissolved in 67% acetic acid into a 5 mg ml−1 stock solution and stored long-term at –20 °C. The working solution was made by diluting the stock solution with phosphate-buffered saline containing 1% bovine serum albumin and 0.05% Tween-20. Each sample was tested in duplicate. To reduce the interference from nonspecific signals due to the passive absorption of various antibodies in plasma to 96-well microplates, a specific binding index was introduced to express the relative levels of circulating antibodies against gliadin-derived antigen (AGA). The specific binding index (SBI) was calculated as follows: SBI = [ODgliadin − ODNC]/[ODmaize – ODNC].

Testing of AGAs

The plasma AGAs were assayed using commercially available kits for both IgG and IgA against the full-length native gliadin molecules (Omega Diagnostics, Cambridge, UK). All the assays were performed according to the manufacturer’s instructions (http://www.omegadiagnostics.com/). The OD reading of each sample was normalized to the mean OD reading of four-well standards provided for qualitative testing.

Data analysis

The Kolmogorov–Smirnov test failed to show a normal distribution of AGDA levels in both the patient and control groups (Supplementary Table 2), so the Mann–Whitney U-test was applied to examine the differences in AGDA levels and AGA levels between the two groups. Owing to multiple testing, the Bonferroni correction was applied to reduce the type I errors and P < 0.006 was considered to be statistically significant. The receiver operating characteristic curve analysis was applied to calculate the area under the receiver operating characteristic curve (AUC) with calculation of the ELISA sensitivity against a specificity of ≥55%. Linear regression was applied to examine which antipsychotic drugs might affect the secretion of AGDA levels in both the patient and control groups (Supplementary Table 2), so the Mann–Whitney U-test was applied to examine the differences in AGDA levels and AGA levels between the two groups. Owing to multiple testing, the Bonferroni correction was applied to reduce the type I errors and P < 0.006 was considered to be statistically significant. The receiver operating characteristic curve analysis was applied to calculate the area under the receiver operating characteristic curve (AUC) with calculation of the ELISA sensitivity against a specificity of ≥55%. Linear regression was applied to examine which antipsychotic drugs might affect the secretion of AGDA levels in both the patient and control groups (Supplementary Table 2), so the Mann–Whitney U-test was applied to examine the differences in AGDA levels and AGA levels between the two groups. Owing to multiple testing, the Bonferroni correction was applied to reduce the type I errors and P < 0.006 was considered to be statistically significant. The receiver operating characteristic curve analysis was applied to calculate the area under the receiver operating characteristic curve (AUC) with calculation of the ELISA sensitivity against a specificity of ≥55%.
Results

Reproducibility of the in-house ELISA
This in-house ELISA had a good reproducibility, in which the inter-assay deviations ranged from 4.6% to 7.5% for AGDA IgA assay and from 9.4% to 16.3% for AGDA IgG assay (Supplementary Table 3).

Levels of circulating AGDA antibodies
As shown in Table 2, patients with schizophrenia had significantly higher levels of plasma anti-AAQ6C IgG than control subjects (Z = -4.65, P < 0.001), but significantly lower levels of IgG antibodies against AL1G1 (Z = -4.65, P < 0.001) AL2G1 (Z = -8.72, P < 0.001), AL2G2 (Z = -6.01, P < 0.001), ABO3a (Z = -6.37, P < 0.001) and ABO3b (Z = -5.32, P < 0.001). The circulating AGDA IgA levels were all significantly lower in the patient group than the control group (Table 3). Exclusion analysis revealed that the male patients were more likely to contribute to altered AGDA levels in the circulation than the female patients (Supplementary Tables 4 and 5).

Levels of circulating AGAs
As shown in Table 4, there was no significant difference in plasma AGA IgG levels between the patient group and the control group (Z = -0.31, P = 0.757). Consistent with the direction of previous studies, a nonsignificant increase in plasma AGA IgA levels was observed in patients with schizophrenia (Z = -0.22, P = 0.825).

Receiver operating characteristic curve analysis
Receiver operating characteristic curve analysis revealed that at a specificity of > 95% (Table 5), five assays had a sensitivity of > 20%, including anti-AAQ6C IgG assay (20.4%, AUC = 0.65), anti-AL2G1 IgG assay (30.7%, AUC = 0.76), anti-AL2G2 IgA assay (20.2%, AUC = 0.71), anti-ABO3a IgA assay (40.0%, AUC = 0.87) and anti-ABO3b IgA assay (35.8%, AUC = 0.81).

Effects of antipsychotic medication on antibody secretion
Linear regression analysis demonstrated that quetiapine was the only antipsychotic drug significantly associated with elevated levels of IgG against AAQ6B (adjusted r^2 = 0.065, t = 3.13, P = 0.002), and eight other antipsychotic drugs did not show a significant association with AGDA IgG levels (Supplementary Table 6); the secretion of AGDA IgA antibodies and AGAs did not appear to be influenced by antipsychotic medication (Supplementary Tables 7 and 8). Fisher’s combining probability test revealed that none of the nine antipsychotic drugs listed in Supplementary Table 1 was significantly associated with the levels of total antibodies against each gliadin-derived antigen in this study (Supplementary Tables 6 and 7).

Correlation between AGDA antibodies and AGAs
Multivariate linear regression analysis revealed a significant correlation between AGA IgG levels and AGDA IgG levels (Supplementary Tables 9 and 10), in which anti-AL1G1 IgG level was the best predictor of AGA IgG level out of all AGDA IgG antibodies tested in the control group (Standardized β = 0.20, P = 0.004), while anti-AAQ6C IgG level was the most significantly correlated to AGA IgG levels in the patient group (Standardized β = 0.17, P = 0.037).

Discussion
This study was undertaken to compare circulating AGDA levels between patients with schizophrenia and healthy controls. The levels of plasma IgG against γ-gliadin-derived antigen AAQ6C were elevated in patients with schizophrenia when compared with healthy controls (Table 2). It is possible that an immune response to the AAQ6C antigen is associated with a subgroup of schizophrenia patients although additional factors, such as their access to the central nervous system, are likely to determine the potential pathological activities of these antibodies in patients with schizophrenia. It has previously been demonstrated that α2-gliadin-derived peptides may not be immunogenic in schizophrenia but are likely to be immunogenic in CD patients.30 A genome-wide association study revealed that the DQA1*0501/DQB1*0201 alleles that encode HLA-DQ2.5 molecules conferring a
major risk of CD, were significantly less prevalent in schizophrenia cases than healthy controls; therefore, the decreased levels of circulating antibodies against α-gliadin-derived antigens may partially result from the low frequency of the DQA1*0501/DQB1*0201 alleles in the patient group. Against all gliadin-derived peptide fragments tested, circulating levels of all AGDA IgA antibodies were significantly lower in schizophrenia patients than healthy controls (Table 3). Although not uniformly observed, a decrease in global IgA levels has been previously measured in patients with schizophrenia and therefore the decrease in AGDA IgA levels may be related to this observation. The role of gastrointestinal inflammation has recently gained attention in the development of schizophrenia as well as in neurological and psychiatric conditions more generally. A previous study that examined the markers of gut-inflammation in non-IgE-mediated cow’s milk allergy demonstrated that infants with such an allergy had a significant decrease in serum IgA in response to food challenge accompanied by a decrease in a subclass of IgG specific for α-casein and an increase in gastrointestinal inflammation. There is also evidence that circulating IgA has an anti-inflammatory role and decreased IgA levels are commonly found in patients with autoimmune disease. Accordingly, decreased AGDA IgA levels observed in the present study may reflect dysfunction of immune-regulation and inflammatory processes possibly in the gastrointestinal tract.

Several studies, including a meta-analysis, have suggested an association between increased AGAs for native gliadins and schizophrenia. However, the present study failed to show a significant increase in either AGA IgG levels or AGA IgA levels, although a nonsignificant increase in AGA IgA levels was observed in patients with schizophrenia (Table 4). All native gliadin molecules consist of ~300 amino acid residues and are unlikely to survive degradation in the digestive system. It is possible that multiple AGAs recognizing distinct epitopes are different between the case group and the control group. Regression analysis examining the correlation between the AGA IgG and the AGDA IgG suggests that anti-AAQ6C IgG is the most predictive of AGA IgG levels in patients with schizophrenia and that anti-AL2G1 IgG is the most predictive of AGA IgG levels in control subjects, suggesting the existence of differential epitopes bound to AGA antibodies in schizophrenia (Supplementary Tables 9 and 10).

Antipsychotic medication is the first-line treatment of schizophrenia but only 50–60% patients show a good response to antipsychotic drugs. Consequently, there is an urgent need to identify specific biomarkers for the precision treatment of the disease. Of eight gliadin-derived antigens tested in this study, four showed a sensitivity of >20% for the detection of their corresponding antibodies in plasma (Table 5). These four tests may thus have a potential to serve as biomarkers for identification of a gluten-related subgroup of schizophrenia, which may be useful for the development of precision treatments.

Owing to the nature of sample collection and the corresponding database information, it was not possible to fully control the potential confounding effects of lifestyle factors, such as alcohol consumption, tobacco use and diets, on the outcomes measured in these case–control samples. Although healthy control subjects were screened for psychiatric illness, there was no additional medical information available and therefore other confounding factors cannot be excluded. Furthermore, the clinical information for patients did not contain consistent reference to clinical subtypes of schizophrenia and so clinical or symptomatic associations for circulating AGDA levels cannot be analysed in this cohort. The lack of antipsychotic-free or drug-naive patients and incomplete medication histories mean that a potential effect of antipsychotic medication on the secretion of anti-gluten antibodies cannot be analysed in this cohort. The lack of antipsychotic-free or drug-naive patients and incomplete medication histories mean that a potential effect of antipsychotic medication on the secretion of anti-gluten antibodies cannot be ruled out, which is a major limitation of this study. Fisher’s combining probability test, however, failed to detect a significant association between antipsychotic medication and circulating anti-gliadin antibody levels (Supplementary Tables 6–8), suggesting that antipsychotic drugs may not significantly affect the secretion of anti-gluten antibodies. Finally, there is an overrepresentation of male subjects in the case group when compared with the control group; the small sample size in females may have underpowered the test for gender differences in antibody secretion.

In summary, this preliminary study demonstrates that altered AGDA levels in the circulation are associated with schizophrenia and could serve as biomarkers for the identification of a schizophrenia subgroup that may need an alternative therapy or precision treatment. Further investigations with clinical trials should be carried out to explore this possibility.

### Table 5. ROC curve analysis of plasma anti-gluten antibodies in schizophrenia

| Antibody test | Specificity (%) | Sensitivity (%) | AUC  | s.e. | P    | 95% CI     |
|---------------|-----------------|----------------|------|------|------|------------|
| IgG           |                 |                |      |      |      |            |
| AAQ6A         | 95.1            | 6.0            | 0.54 | 0.03 | 0.243| 0.48–0.53  |
| AAQ6B         | 95.0            | 8.4            | 0.58 | 0.03 | 0.008| 0.52–0.64  |
| AAQ6C         | 95.1            | 20.4           | 0.65 | 0.03 | <0.001| 0.59–0.70  |
| AL1G1         | 95.0            | 4.2            | 0.64 | 0.03 | <0.001| 0.58–0.70  |
| AL2G1         | 95.1            | 30.7           | 0.76 | 0.03 | <0.001| 0.71–0.81  |
| AL2G2         | 95.1            | 15.0           | 0.68 | 0.03 | <0.001| 0.63–0.73  |
| ABO3a         | 95.3            | 13.8           | 0.69 | 0.03 | <0.001| 0.64–0.75  |
| ABO3b         | 95.3            | 7.5            | 0.66 | 0.03 | <0.001| 0.61–0.72  |
| Gliadin       | 95.1            | 5.4            | 0.51 | 0.03 | 0.757| 0.43–0.55  |

| IgA           |                 |                |      |      |      |            |
|---------------|-----------------|----------------|------|------|------|------------|
| AAQ6A         | 95.1            | 18.0           | 0.72 | 0.03 | <0.001| 0.67–0.77  |
| AAQ6B         | 95.1            | 6.6            | 0.59 | 0.03 | 0.002| 0.53–0.65  |
| AAQ6C         | 95.0            | 16.4           | 0.70 | 0.03 | <0.001| 0.65–0.75  |
| AL1G1         | 95.0            | 10.9           | 0.62 | 0.03 | <0.001| 0.57–0.68  |
| AL2G1         | 95.0            | 14.5           | 0.71 | 0.03 | <0.001| 0.66–0.76  |
| AL2G2         | 95.0            | 20.2           | 0.71 | 0.03 | <0.001| 0.66–0.76  |
| ABO3a         | 95.0            | 40.0           | 0.87 | 0.02 | <0.001| 0.83–0.91  |
| ABO3b         | 95.0            | 35.8           | 0.81 | 0.02 | <0.001| 0.76–0.85  |
| Gliadin       | 95.1            | 6.6            | 0.49 | 0.03 | 0.825| 0.44–0.55  |

Abbreviations: AUC, area under the ROC curve; CI, confidence interval; Ig, immunoglobulin; ROC, receiver operating characteristic.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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REFERENCES
1. Tandon R, Gaebel W, Barch DM, Bustillo J, Gur RE, Heckers S et al. Definition and description of schizophrenia in the DSM-V. Schizophr Res 2013; 150: 3–10.
2. Maran M, Grent ’ t-Jong T, Uhihaa PJ. Electrophysiological insights into connectivity anomalies in schizophrenia: a systematic review. Neuropsychiatr Electrophysiol 2016; 2: 6.
3. Wobrock T, Schneider M, Kadovic D, Schneider-Axmann T, Ecker UKH, Retz W et al. Reduced cortical inhibition in first-episode schizophrenia. Schizophr Res 2008; 105: 252–261.
4. Perry T, Buchanan J, Kish S, Hansen S. y-Aminobutyric-acid deficiency in brain of schizophrenic patients. Lancet 1979; 313: 237–239.
5. Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Selby JP, Bowers M et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 1998; 155: 761–767.
6. van Os J. “Schizophrenia” does not exist. BMJ 2016; 352: i375.
7. Solanki RK, Singh P, Munshi D. Current perspectives in the treatment of resistant schizophrenia. Acta Psychiatr Scand 2015; 132: 20–31.
8. Jablensky A, Sartorius N, Ernberg G, Anker M, Korten A, Cooper JE et al. Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. Psychol Med Monogr Suppl 1992; 20: 1–97.
9. Dohan FC. Wheat “consumption” and hospital admissions for schizophrenia during World War II. A preliminary report. Am J Clin Nutr 1966; 18: 7–10.
10. Dohan FC, Grasberger JC. Relapsed schizophrenia: earlier discharge from the hospital after cereal-free, milk-free diet. Am J Psychiatry 1973; 130: 685–688.
11. Dohan FC, Grasberger JC, Lowell FM, Johnston HT, Arbogast AW. Relapsed schizophrenics: more rapid improvement on a milk- and cereal-free diet. Br J Psychiatry J Ment Sci 1969; 115: 595–596.
12. Jackson J, Eaton W, Cascella N, Fasano A, Santora D, Sullivan K et al. Gluten sensitivity and relationship to psychiatric symptoms in people with schizophrenia. Schizophr Res 2014; 159: 539–542.
13. Kalaydjian AE, Eaton W, Cascella N, Fasano A. The gluten connection: the association between schizophrenia and celiac disease. Acta Psychiatr Scand 2006; 113: 82–90.
14. Lionetti E, Leonardi S, Franzonello C, Mancardi M, Ruggieri M, Catassi C. Gluten psychosis: confirmation of a new clinical entity. Nutrients 2015; 7: 5532–5539.
15. Eaton WW, Chen L-Y, Dohan FC, Kelly DL, Cascella N. Improvement in psychotic symptoms after a gluten-free diet in a boy with complex autoimmune illness. Am J Psychiatry 2015; 172: 219–222.
16. Muller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. Front Neurosci 2015; 9: 372.
17. Schwiefer L, Larsson MK, Skogh E, Kegel ME, Orhan F, Abdelmoaty S et al. Increased levels of IL-6 in the cerebrospinal fluid of patients with chronic schizophrenia—significance for activation of the kynurenine pathway. Psychiatry Neurosci 2015; 40: 126–133.
18. Bloomfield PS, Selvaraj S, Veronese M, Rizzo G, Bertoldo A, Owen DR et al. Microglial activity in people at ultra high risk of psychosis and in schizophrenia: an [(11)C]CPPBR28 PET brain imaging study. Am J Psychiatry 2016; 173: 44–52.
19. Dickerson F, Stallings C, Origni A, Schroeder J, Katsafanas E, Schweinfurth L et al. Inflammatory markers in recent onset psychosis and chronic schizophrenia. Schizophr Bull 2016; 42: 134–141.
20. Cascella NG, Kryszak D, Bhatti B, Gregory P, Kelly DL, McEvoy JP et al. Prevalence of celiac disease and gluten sensitivity in the United States clinical antipsychotic trials of intervention effectiveness study population. Schizophr Bull 2011; 37: 94–100.
21. Dickerson F, Stallings C, Origni A, Vaughan G, Khushalani S, Leister F et al. Markers of gluten sensitivity and celiac disease in recent-onset psychosis and multi-episode schizophrenia. Biol Psychiatry 2010; 68: 100–104.

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22. Jin S-Z, Wu N, Xu Q, Zhang X, Ju G-Z, Law MH et al. A study of circulating gliadin antibodies in schizophrenia among a Chinese population. Schizophr Bull 2012; 38: 514–518.
23. Okusaga O, Yolken RH, Langenberg P, Sleemi A, Kelly DL, Vaswani D et al. Elevated gliadin antibody levels in individuals with schizophrenia. World J Biol Psychiatry 2013; 14: 509–515.
24. Reichelt KL, Landmark J. Specific IgA antibody increases in schizophrenia. Biol Psychiatry 1995; 37: 410–413.
25. Lachance LR, McKenzie K. Biomarkers of gluten sensitivity in patients with non-affective psychosis: a meta-analysis. Schizophr Res 2014; 152: 521–527.
26. International Schizophrenia Consortium, Purcell SM, Wray NR, Stone JL, Visscher PM, O’Donovan MC et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 2009; 460: 748–752.
27. Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe’er I et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 2009; 460: 753–757.
28. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014; 511: 421–427.
29. Schwerdt E, Kahlenberg F, Sack U, Richter T, Stern M, Conrad K et al. Serological assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. Clin Chem 2004; 50: 2370–2375.
30. Samaroo D, Dickerson F, Kassada DR, Green PFR, Brian C, Yolken RH et al. Novel immune response to gluten in individuals with schizophrenia. Schizophr Res 2010; 118: 248–255.
31. Shan L, Qiao S-W, Arentzen-Hansen M, Mollberg Ø, Gray GM, Solid LM et al. Identification and analysis of multivalent proteolytically resistant peptides from gluten: implications for celiac sprue. J Proteome Res 2005; 4: 1732–1741.
32. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res 2003; 31: 3784–3788.
33. Sóllner J, Heinzel A, Summer G, Fechete R, Stipkovits L, Szathmary S et al. Concept and application of a computational vaccinology workflow. Immunome Res 2010; 6 (Suppl 2): 57.
34. Wang P, Sidney J, Kim Y, Sette A, Lund O, Nielsen M et al. Peptide binding predictions for HLA DR, DP and DQ molecules. BMC Bioinformatics 2010; 11: 506.
35. Elston RC. On Fisher’s method of combining P-values. Biom J 1991; 33: 339–345.
36. Delisi LE, Weinberger DR, Potkin S, Necker LS, Shilng DJ, Wyatt RJ. Quantitative determination of immunoglobulins in CSF and plasma of chronic schizophrenic patients. Br J Psychiatry J Ment Sci 1981; 139: 513–518.
37. Tiwari SG, Lal N, Trivedi JK, Sayeed J, Bahauguna LM. Immunoglobulin patterns in schizophrenia and bipolar disorder. World J Biol Psychiatry 2008; 9: 518–522.
38. Prevalence of celiac disease and gluten sensitivity in the United States clinical antipsychotic trials of intervention effectiveness study population. Schizophr Res 2011; 139: 513–518.
39. Russell MW, Reinholdt J, Kilian M. Anti-inflammatory activity of human IgA antibodies and their Fabo fragments: inhibition of IgG-mediated complement activation. Eur J Immunol 1989; 19: 2243–2249.
40. Jacob CMA, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC. Autoimmunity in IgA deficiency: revisiting the role of IgA as a silent housekeeper. J Clin Immunol 2008; 28: 56–61.

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