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Original Article

Seroreactive marker for inflammatory bowel disease and associations with antibodies to dietary proteins in bipolar disorder

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Objectives: Immune sensitivity to wheat glutens and bovine milk caseins may affect a subset of individuals with bipolar disorder. Digested byproducts of these foods are exorphins that have the potential to impact brain physiology through action at opioid receptors. Inflammation in the gastrointestinal (GI) tract might accelerate exposure of food antigens to systemic circulation and help explain elevated gluten and casein antibody levels in individuals with bipolar disorder.

Methods: We measured a marker of GI inflammation, anti-*Saccharomyces cerevisiae* antibodies (ASCA), in non-psychiatric controls (n = 207), in patients with bipolar disorder without a recent onset of psychosis (n = 226), and in patients with bipolar disorder with a recent onset of psychosis (n = 38). We compared ASCA levels to antibodies against gluten, casein, Epstein–Barr virus (EBV), herpes simplex virus 1 (HSV-1), influenza A, influenza B, measles, and *Toxoplasma gondii*.

Results: Elevated ASCA conferred a 3.5–4.4-fold increased odds ratio of disease association (age-, race-, and gender-corrected multinomial logistic regressions, $p \leq 0.00001$) that was independent of type of medication received. ASCA correlated with food antibodies in both bipolar disorder groups ($R^2 = 0.29–0.59$, $p \leq 0.0005$), and with measles and *T. gondii* immunoglobulin G (IgG) in the recent onset psychosis bipolar disorder group ($R^2 = 0.31–0.36$, $p \leq 0.004–0.01$).

Conclusions: Elevated seropositivity of a GI-related marker and its association with antibodies to food-derived proteins and self-reported GI symptoms suggest a GI comorbidity in at least a subgroup of individuals with bipolar disorder. Marker seroreactivity may also represent part of an overall heightened activated immune state inherent to this mood disorder.

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There currently exists a renewed interest in the role of wheat glutens and bovine milk caseins in the etiology and pathophysiology of psychiatric disorders (1–14). F. Curtis Dohan (15–20) originally proposed that these food antigens might impact schizophrenia, based on observations of celiac disease in individuals with schizophrenia and on correlations between wartime wheat deficits and diminished schizophrenia hospitalization rates. Several recent studies indicate that individuals with mood disorders may also be affected by food-related immune activation, particularly during the mania and psychosis-associated phases of the disease (3, 5, 8). The mechanism governing how food antigen peptides might impact the brain and behavior is not known, but is thought to involve the digestion of glutens and caseins into small peptides that are opioid receptor ligands (21–28). For

these peptides to access the brain, they must first be absorbed across gastrointestinal (GI) epithelial and endothelial barriers and enter into systemic circulation.

We recently reported that a marker of GI inflammation was elevated and correlated with levels of antibodies to food antigens in individuals with schizophrenia compared to controls (6). In particular, individuals who were in the early stages of disease and/or who were medication-naïve had the highest levels of GI inflammation. Data from serological, gene expression, and imaging studies indicate that inflammation may be an important pathology in individuals with affective disorders (29–38). Defining a direct relationship between mood disorder symptoms and cytokine dysregulation has been difficult, however, given the fluctuating nature of mood cycles, the anti-inflammatory effects of mood disorder drugs, and the natural dynamic process of inflammatory responses (30). Detection of a localized source of inflammation in the GI tract could help to explain disease-associated systemic inflammation, because a permeable GI tract can lead to translocation of gut microbiota into circulation, thus causing a more generalized inflammatory state (39).

To determine if inflammation present in the GI tract might be a comorbid risk factor for bipolar disorder, we measured anti-*Saccharomyces cerevisiae* antibodies (ASCA) in individuals with bipolar disorder without a recent onset of psychosis, in individuals with bipolar disorder with a recent onset of psychosis and in controls with no history of psychiatric disorders. Measures of serological ASCA are used clinically to aid and differentiate the diagnosis of inflammatory bowel diseases including ulcerative colitis and Crohn's disease (40–44). Our objectives were to evaluate if this marker of GI inflammation was: (i) elevated in disease groups compared to controls, (ii) accompanied by an anti-food antigen immune response, (iii) accompanied by an anti-microbial pathogen immune response, (iv) associated with recent onset or lifetime psychosis, (v) prevalent during a particular phase of the bipolar disorder cycle, and (vi) correlated with the presence of self-reported GI symptoms.

Materials and methods

Study participants

Study participants were recruited from the Baltimore, MD, USA metro area. One objective of our study design was to enable comparisons between recently diagnosed individuals and those individuals who had had their disease for greater amounts of time. For measures of inflammatory indices that are known to fluctuate over time, the ability to compare individuals in different stages of disease is especially pertinent (6, 8, 9, 32, 45). Toward this end, we recruited 226 individuals diagnosed with bipolar disorder without a recent onset of psychosis, 38 individuals with bipolar disorder and a recent onset of psychosis, and 207 individuals who did not have a history of psychiatric disorders from inpatient and outpatient treatment sites and published announcements. These individuals comprise a cohort that has been previously evaluated in a number of studies (3, 4, 8). The methods for identifying and characterizing individuals of the diagnostic groups according to criteria defined by DSM-IV also have been previously described (3, 4, 8). The DSM-IV diagnoses and sample sizes for the two bipolar disorder groups are listed in Table 1.

Basic demographic data of the control and bipolar disorder groups are shown in Table 2. Diagnostic groups differed significantly in age and race and these variables were included in the multivariate analyses described below.

For the 226 individuals with bipolar disorder without a recent onset of psychosis, inclusion criteria were: (i) diagnosis of type I or type II bipolar disorder or bipolar disorder not otherwise specified according to DSM-IV criteria (46); (ii) age $\geq 18 \leq 65$ years; (iii) absence of primary diagnosis of substance abuse or dependence over the past three months; (iv) absence of any history of intravenous substance abuse; (v) absence of mental retardation; and (vi) absence of any clinically significant medical disorder that would affect cognitive performance such as history of encephalitis or serious head trauma, or any other significant neurological disorder of the central nervous system.

Table 1. Diagnostic subtypes of bipolar disorder (BP) in the study population

Diagnosis	BP without ROP (n = 226) n (%)	BP with ROP (n = 38) n (%)
BP-I, most recent episode manic	89 (39.4)	20 (52.6)
BP-I, most recent episode depressed	53 (23.5)	10 (26.3)
BP-I, most recent episode mixed	59 (26.1)	6 (15.8)
BP-NOS	1 (0.4)	0 (0)
BP-II	24 (10.6)	2 (5.3)

BP-I = bipolar I disorder; BP-II = bipolar II disorder; NOS = not otherwise specified; ROP = recent onset psychosis.

Table 2. Demographic information

	Controls (n = 207)	Bipolar disorder without ROP (n = 226)	Bipolar disorder with ROP (n = 38)
Duration of illness, years, mean ± SEM	–	20.03 ± 0.82	0.90 ± 0.42
Age, years, mean ± SEM	32.07 ± 0.80	37.81 ± 0.84[a]	26.69 ± 1.31[b]
Males, n (%)	56 (27.1)	68 (30.1)	12 (31.6)
African American, n (%)	66 (31.9)	48 (21.2)[c]	9 (23.7)
RBANS score, mean ± SEM	88.80 ± 0.82	79.23 ± 0.95[d]	78.63 ± 2.02[e]
PANSS score, mean ± SEM	–	69.35 ± 0.95	70.71 ± 2.47
YMRS score, mean ± SEM	–	13.40 ± 0.64	–
HRSD score, mean ± SEM	–	19.97 ± 0.71	–
History of psychotic features, n (%)	0 (0)	170 (75.2)	38 (100)

HRSD = Hamilton Rating Scale for Depression; PANSS = Positive and Negative Syndrome Scale; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; ROP = recent onset psychosis; SEM = standard error of the mean; YMRS = Young Mania Rating Scale.
[a] $t = -4.94$, $p \leq 0.00001$.
[b] $t = 2.77$, $p \leq 0.006$.
[c] $\chi^2 = 6.31$, $p \leq 0.012$.
[d] $t = 7.54$, $p \leq 0.00001$.
[e] $t = 4.80$, $p \leq 0.00001$.

While these individuals did not have a recent onset of psychosis defined as an onset of psychotic symptoms for the first time within the past two years, 75.2% had a history of past psychosis, as noted in Table 2.

Additional inclusion criteria for the 38 individuals with bipolar disorder and a recent onset of psychosis were the onset of psychotic symptoms for the first time within the past 24 months, defined as the presence of a positive psychotic symptom of at least moderate severity that lasted through the day for several days or that occurred several times a week; and age $\geq 18 \leq 45$ years.

The 207 individuals without a history of psychiatric disorder were screened to rule out current or past psychiatric disorders with the Structured Clinical Interview for DSM-IV Axis I Disorders (47). Control participants were aged $\geq 20 \leq 60$ years. Exclusion criteria were: (i) any history of intravenous substance abuse; (ii) mental retardation; (iii) having a clinically significant medical disorder that would affect cognitive performance; or (iv) current substance abuse that occurred within the last three months. Immunoassay data from this control group have been previously reported (6).

At the time of interview, cognitive function was evaluated with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) Form A (48) and psychiatric symptoms rated according to the Positive and Negative Syndrome Scale (PANSS) (49). For the main bipolar disorder group, symptom severity was assessed with the Hamilton Rating Scale for Depression (HRSD) and the Young Mania Rating Scale (YMRS) (50, 51). Mean scores for these tests are shown in Table 2. HRSD and YMRS data were not available for the recent onset psychosis bipolar disorder group.

During the interview, clinical information was also gathered, and individuals were asked to report any conditions related to their current GI health. Relevant conditions included constipation, Crohn's disease, diarrhea, gastroesophageal reflux disease (GERD), irritable bowel syndrome, lactose intolerance, Norwalk virus, pancreatitis, ulcers, any abdominal surgery, and any abdominal pain. For both bipolar disorder groups, GERD was the most frequently reported GI condition, and these data are shown in Table 3.

Blood samples were obtained by venipuncture, and sera were separated and assessed for antibodies to the antigens in the assays described below.

The studies were approved by the Institutional Review Boards (IRBs) of the Sheppard Pratt Health System and the Johns Hopkins Medical Institution following established guidelines. All participants provided written informed consent after study procedures were explained. The work described was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Laboratory procedures

Anti-*Saccharomyces cerevisiae* immunoglobulin G (IgG) antibodies (ASCA) were measured according to the manufacturer's protocol using a commercially available kit (Orgentec, Mainz, Germany). IgG antibodies to bovine milk casein

Table 3. Gastrointestinal conditions self-reported by individuals with bipolar disorder

	Bipolar disorder without ROP (n = 84)[a]	Bipolar disorder with ROP (n = 13)[b]
Constipation	11 (13.1)	2 (15.4)
Diarrhea	3 (3.6)	1 (7.7)
Gastroesophageal reflux disease	35 (41.7)	6 (46.2)
Crohn's disease or irritable bowel syndrome	5 (6.0)	1 (7.7)
Ulcers	1 (1.2)	0 (0)
Any abdominal surgery	19 (22.6)	1 (7.7)
Any abdominal pain	4 (4.8)	0 (0)
Other GI conditions	6 (7.1)	2 (15.4)

Values are reported as n (%). GI = gastrointestinal; ROP = recent onset psychosis.
[a]Information regarding absence or presence of GI conditions was collected for n = 165.
[b]Information regarding absence or presence of GI conditions was collected for n = 37.

and wheat gluten were measured by enzyme-linked immunosorbent assays (ELISAs) using previously described methods (6, 7). Whole casein was purchased from Sigma-Aldrich (St. Louis, MO, USA). Whole gluten was extracted from the wheat cultivar Cheyenne as previously described (12). In brief, for both the casein and gluten immunoassays, plate wells were incubated with 100 ng of protein in 50 μL of carbonate buffer (0.05 M carbonate-bicarbonate, pH 9.6; Sigma-Aldrich) overnight at 4°C, and plates were blocked for 1 hour at 37°C with 1% (wt/vol) human serum albumin (Sigma-Aldrich) in phosphate-buffered saline (PBS). Plates were then incubated with samples diluted 1:200 in PBS-Tween 20 for 2 hours at 37°C. Plates were washed and incubated with peroxidase-conjugated goat-anti-human IgG secondary antibodies for 30 min at 37°C (Southern Biotech, Birmingham, AL, USA). A 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) and 0.02% hydrogen peroxide solution (KPL Protein Research Products, Gaithersburg, MD, USA) was added for color development, and absorbance was measured at 405 nm, with a reference wavelength of 490 nm, in an automated microtiter plate reader (Molecular Devices, Menlo Park, CA, USA).

Commercially available ELISA kits for measuring Epstein–Barr virus (EBV) IgG, influenza A IgG, influenza B IgG, measles IgG, and *T. gondii* IgG were purchased from IBL America (Minneapolis, MN, USA) and/or IBL International GmbH (Hamburg, Germany). Herpes simplex virus 1 (HSV-1) kits were purchased from Focus Diagnostics (Cypress, CA, USA). IgG levels produced in response to these infectious disease agents had been previously measured for a series of studies at the Stanley Division at Johns Hopkins. IgG levels from these assays have also been previously analyzed for the control individuals in a comparison of these subjects with individuals who had schizophrenia (6).

Statistical analyses

Plate-to-plate variation was corrected by control mean-normalizing each plate so that the samples from control individuals on any particular plate had a value of '1', as previously described (6, 7). Quantitative levels of antibodies to ASCA were compared among groups with ANOVAs, followed by Bonferroni post hoc analyses and *t*-tests. ASCA seropositivity levels were defined based on 90% control values. Multinomial logistic regressions were used to establish age-, sex- and race-corrected odds ratios of ASCA seropositivity for disease association. Multiple linear regressions corrected for age, sex and race were implemented to test for inter-correlations of ASCA, food antigen and infectious disease antigen IgG antibody levels. Age, RBANS, PANSS, YMRS, and HRSD scores were also evaluated separately for correlations with IgG levels in these multiple linear regressions. Each bipolar disorder group was further stratified according to sex, race, the presence of self-reported GI symptoms, the most recent phasic cycle (manic, mixed, or depressive), and the presence or absence of past psychotic features; and the same statistical analyses were applied. Regression coefficient values that equaled or exceeded 0.15 and p-values <0.05 were considered significant. Statistical analyses were performed with STATA version 12 (STATA Corp LP, College Station, TX, USA).

Results

Both bipolar disorder groups had significantly higher ASCA levels than controls (Fig. 1A) (ANOVA, $F = 18.51$, $p \leq 0.00001$; post hoc Bonferroni p-value range: 0.0001–0.001). ASCA seropositivity was associated with an odds ratio for disease of 4.40 in bipolar disorder without a recent onset of psychosis [95% confidence interval (CI): 2.54–7.62, $p \leq 0.00001$] and of 3.52 in bipolar disorder with

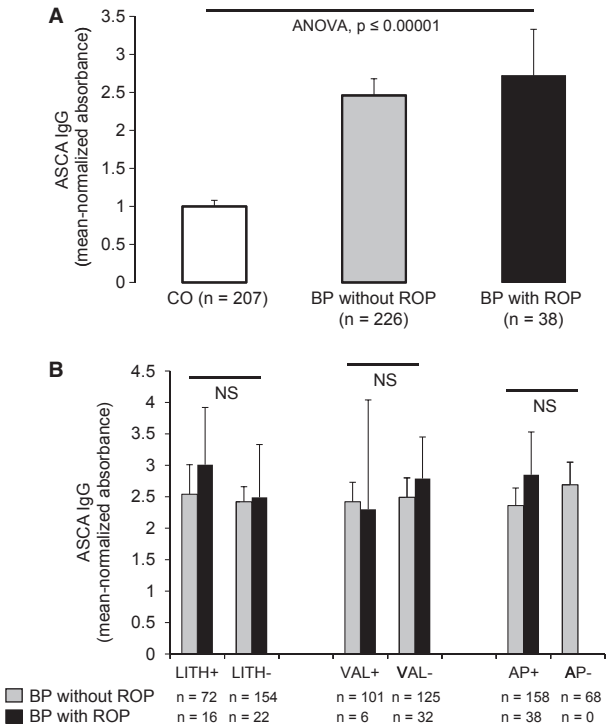

Fig. 1. Quantitative anti-*Saccharomyces cerevisiae* antibody (ASCA) immunoglobulin G (IgG) levels according to diagnosis and medication status. (**A)** ASCA IgG levels were significantly elevated in bipolar disorder (BP) without a recent onset of psychosis (ROP) and BP with ROP compared to controls (CO). (**B)** ASCA IgG levels were not significantly different between individuals who were medication-positive (+) and medication-negative (−). AP = antipsychotic; LITH = lithium; VAL = valproate.

recent onset psychosis (95% CI: 1.48–8.39, $p \leq 0.00001$) as compared to controls. ASCA levels within diagnostic groups were not significantly different between male and female individuals or between racial groups (African American and Caucasian/other).

To evaluate if ASCA levels were affected by psychiatric medications, we further subdivided the bipolar disorder groups according to current receipt of lithium, valproate or antipsychotic medication (Fig. 1B). No significant differences in ASCA measures were found between receipt or not of each of these medication types (Fig. 1B). Of note, all individuals in the bipolar disorder recent onset psychosis group were taking antipsychotic medications at the time of the study visit.

When individuals with bipolar disorder without a recent onset of psychosis were broken down according to the absence or presence of past psychotic features, no significant differences in ASCA levels were detected. There were also no statistically significant differences associated with ASCA with respect to lifetime psychosis when both bipolar disorder groups were pooled.

To determine if there were correlations between ASCA levels and antibodies to food and microbial antigens, multiple linear regression models incorporating age, sex and race were implemented. Anti-casein IgG levels have previously been reported for only a subset of this entire cohort (8), so we first measured IgG to casein and to gluten in the entire set. Levels of IgG to food antigens were significantly increased in the two bipolar disorder groups compared to controls (casein: ANOVA, $F = 16.03$, $p \leq 0.00001$; post hoc Bonferroni $p \leq 0.0001$; gluten: ANOVA, $F = 18.35$, $p \leq 0.00001$; post hoc Bonferroni $p \leq 0.0001$). In regression models, ASCA levels were highly correlated to IgG to casein and gluten food antigens in both bipolar disorder groups, but not in controls (Fig. 2) (p-value range: 0.00001–0.0005). Casein and gluten IgGs were highly inter-correlated in all three diagnostic groups (Table 4). ASCA was generally not correlated with IgG antibodies directed at four of the microbial pathogens (EBV, HSV-1, influenza A, and influenza B), but in the recent onset psychosis bipolar disorder group, ASCA levels were significantly associated with IgG to measles and to *T. gondii* (Table 4). When these groups were broken down according to sex, we found that it was only in female individuals that these microbial pathogen correlations were significant (p-value range: 0.004–0.0008).

ASCA was not correlated to age, cognitive scores, or symptom severity scores on the RBANS, PANSS, YMRS, or HRSD assessments.

The bipolar disorder groups were also subdivided according to type of most recent episode: manic, depressed, or mixed. In bipolar disorder without a recent onset of psychosis, ASCA levels were correlated to food antigen IgG in all three phase subgroups (Table 5) (p-value range: 0.00001–0.001). In the bipolar disorder with recent onset psychosis group, however, it was only in the manic subgroup that ASCA and the food antigen IgG were correlated. We also further investigated IgG to the microbial pathogens measles and *T. gondii*, and found that ASCA was strongly correlated to measles IgG in the manic and mixed subgroups of the bipolar disorder without recent onset psychosis group and in the manic subgroup of the bipolar disorder with recent onset psychosis group (Table. 5) (p-value range: 0.0001–0.04). ASCA and *T. gondii* IgG were not correlated in any of these subgroup comparisons.

We then examined ASCA levels and correlations to food and microbial antigens according to self-reported GI-related symptomatology. Levels of ASCA were not significantly different between those who reported GI-related conditions and

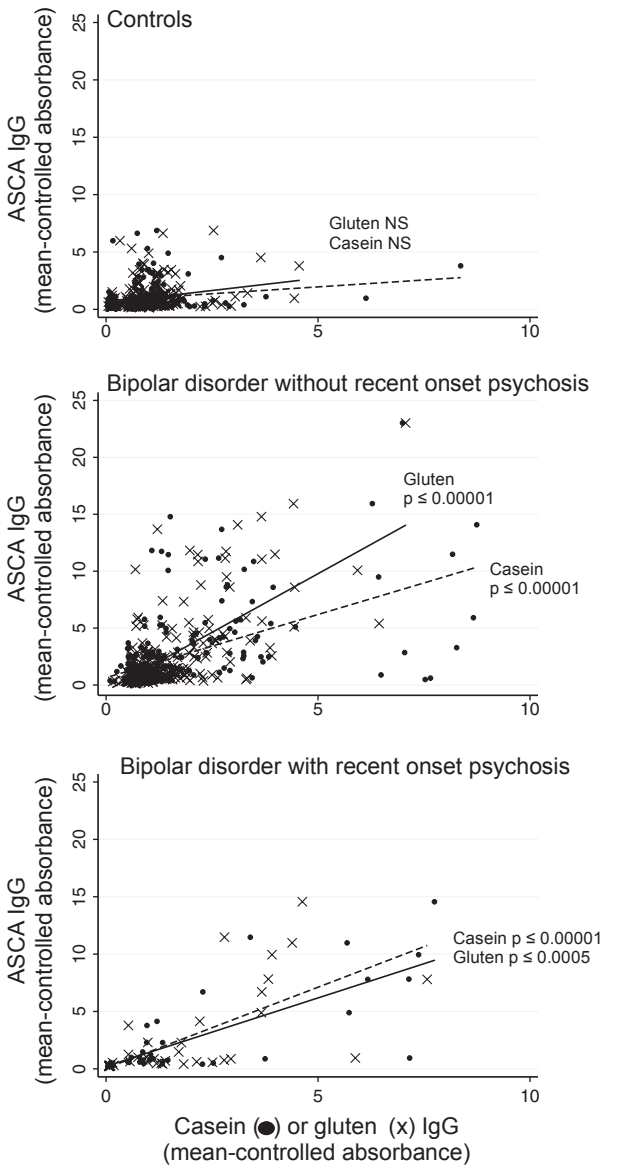

Fig. 2. Anti-*Saccharomyces cerevisiae* antibody (ASCA) immunoglobulin G (IgG) correlates with food antigen IgG in the bipolar disorder groups, but not controls. Gluten data points are shown with an '×' and a solid regression line; casein data points are shown with a closed '●' and a dashed regression line. Multiple linear regressions corrected for age, sex and race were used to generate the listed p-values. NS = not significant.

those who did not, although a trend for higher ASCA in GI-symptom positive individuals was apparent [bipolar disorder without a recent onset of psychosis: *t*-test, $t = -1.3587$, $p \leq 0.08$, mean ± standard error of the mean (SEM) = 1.59 ± 0.28 for GI-positive (n = 84) versus 1.17 ± 0.13 for GI-negative (n = 81); bipolar disorder with a recent onset of psychosis, $t = -0.93$, $p \leq 0.17$, 3.56 ± 1.31 for GI-positive (n = 24), 2.32 ± 0.67 for GI-negative (n = 13)]. In bipolar disorder without a recent onset of psychosis, only in the subgroup reporting GI symptoms were

Table 4. Inter-correlations of gastrointestinal inflammation, food antigen immunoglobulin G (IgG) and microbial pathogen IgG

	Controls	Bipolar disorder without ROP	Bipolar disorder with ROP
Food antigens			
ASCA:Casein			
n	207	226	38
R^2	0.04	**0.29**	**0.59**
p	0.12	**0.00001**	**0.00001**
ASCA:Gluten			
n	207	226	38
R^2	0.07	**0.44**	**0.45**
p	0.005	**0.00001**	**0.0005**
Casein:Gluten			
n	207	226	38
R^2	**0.71**	**0.51**	**0.77**
p	**0.00001**	**0.00001**	**0.00001**
Microbial pathogens			
EBV			
n	207	226	38
R^2	0.008	0.03	0.03
p	0.81	0.13	0.91
HSV-1			
n	207	220	12
R^2	0.008	0.04	0.22
p	0.81	0.09	0.74
Influenza A			
n	207	226	38
R^2	0.01	0.04	0.13
p	0.67	0.04	0.30
Influenza B			
n	207	226	38
R^2	0.008	0.04	0.16
p	0.82	0.08	0.21
Measles			
n	207	225	38
R^2	0.01	0.11	**0.31**[a]
p	0.73	0.0001	**0.01**
T. gondii			
n	207	222	38
R^2	0.008	0.08	**0.36**[b]
p	0.8	0.002	**0.004**

Bolded entries indicate statistically significant regression ($p \leq 0.05$).
EBV = Epstein–Barr virus; HSV-1 = herpes simplex virus 1; ROP = recent onset psychosis; *T. gondii* = *Toxoplasma gondii*.
[a]Females: $R^2 = 0.45$, $p \leq 0.004$; males: not significant.
[b]Females: $R^2 = 0.53$, $p \leq 0.0008$; males: not significant.

significant correlations of ASCA with casein and gluten IgGs maintained (Fig. 3) (casein: $R^2 = 0.39$, $p \leq 0.00001$; gluten: $R^2 = 0.59$, $p \leq 0.00001$). In the bipolar disorder with recent onset psychosis group, ASCA levels were correlated with food antigen IgG in both those who reported GI symptoms and those who did not (R^2 range: 0.58–0.73, p-value range: 0.001–0.05).

Discussion

In this study, we found that a marker of inflammatory bowel disease, ASCA, was elevated in bipolar

Table 5. Inter-correlations of gastrointestinal inflammation, food antigen immunoglobulin (IgG) and microbial pathogen IgG according to bipolar disorder cycle phase

	Bipolar disorder without ROP			Bipolar disorder with ROP		
	Mania	Depressed	Mixed	Mania	Depressed	Mixed
n[a]	89	53	59	20	10	6
Food antigens						
ASCA:Casein						
R^2	**0.35**	**0.31**	**0.47**	**0.75**	0.28	0.99
p	**0.00001**	**0.001**	**0.00001**	**0.0002**	0.74	0.16
ASCA:Gluten						
R^2	**0.44**	**0.60**	**0.35**	**0.80**	0.42	0.87
p	**0.00001**	**0.00001**	**0.0001**	**0.00001**	0.52	0.52
Casein:Gluten						
R^2	**0.33**	**0.72**	**0.55**	**0.88**	**0.88**	0.97
p	**0.00001**	**0.00001**	**0.00001**	**0.00001**	**0.02**	0.27
Microbial pathogens						
Measles						
R^2	**0.25**	0.14	**0.33**	**0.48**	0.17	0.73
p	**0.0001**	0.14	**0.0002**	**0.04**	0.89	0.71
T. gondii						
R^2	0.06	0.15	0.15	0.35	0.63	0.64
p	0.25	0.12	0.07	0.14	0.22	0.79

Bolded entries indicate statistically significant regression ($p \leq 0.05$).
ROP = recent onset psychosis; *T. gondii* = *Toxoplasma gondii*.
[a]Individuals with a diagnosis of bipolar II disorder or bipolar disorder not otherwise specified were not included in these analyses. Therefore, the total sample size for bipolar disorder without ROP was n = 201 and for bipolar disorder with ROP, the total sample size was n = 36.

disorder with and without a recent onset of psychosis compared to non-psychiatric controls. An ASCA-seropositive status conferred a 3.5–4.4-fold increased odds ratio for disease association. Medication status did not appear to impact measures of this GI marker, as no significant differences in ASCA levels between those receiving and not receiving lithium, valproate or antipsychotics were detected. Bipolar disorder-related ASCA levels were significantly greater than those found previously in schizophrenia (data not shown, ANOVA $p \leq 0.001$) (6). Further studies are required to fully disentangle if the GI locale represents a source of inflammation or if it is a pathology stemming from a generalized state of immune activation intrinsic to bipolar disorder.

We demonstrated a significant disease association of the ASCA marker with immune reactivity to wheat glutens and bovine milk caseins. In particular, these correlations were significant in individuals of the main bipolar disorder group who reported GI symptoms compared to those who did not have GI symptoms. Together, these findings may support hypotheses related to pre-existing, compromised epithelial barriers; however, other explanations are also possible, including a directed interaction of food exorphins with tight junction proteins or epithelial cell transcytosis (52–59). Incomplete digestion of these foods could also generate novel antigens that prime the immune system. We found previously that sera from individuals with bipolar disorder and schizophrenia did not recognize the same epitopes of casein and gluten that were recognized by sera from controls (7, 8, 12). Differential processing of these food proteins may result from alterations in the resident gut microbiota or dysfunction of digestive peptidases. We can speculate that if GI inflammation does indeed cause GI permeability, not only would partially digested gluten and casein peptides enter into the bloodstream, but resident gut microbiota would translocate into systemic circulation. Maes and colleagues (60–62) have repeatedly demonstrated an association of bacterial translocation with major depressive disorder, and we are exploring this hypothesis in schizophrenia (63).

Because individuals in our control group may not be afflicted with the same degree of GI inflammation as cases, the constricted quantitative range of control ASCA values may make it difficult to statistically detect the correlations of ASCA with other variables such as antibodies to food peptides; however, inter-correlations of the food-related antibodies were quite strong in all diagnostic groups. As such, the lack of association of ASCA markers with antibodies to dietary proteins in the controls may reflect mechanisms of immune activation in cases which are alternative to those of the

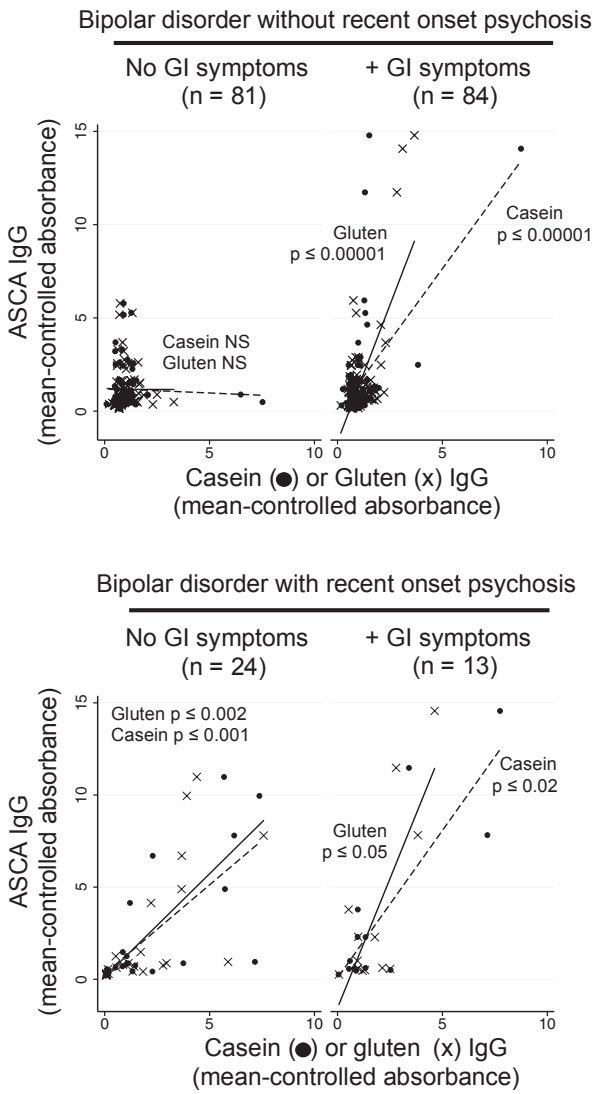

Fig. 3. Correlations of anti-*Saccharomyces cerevisiae* antibody (ASCA) and food antigen immunoglobulin G (IgG) levels according to self-reported symptoms of gastrointestinal (GI) discomfort. IgG against food antigens was correlated to ASCA in individuals with bipolar disorder without a recent onset of psychosis who reported GI symptoms (+GI symptoms), but not in those who did not report these symptoms. In bipolar disorder with a recent onset of psychosis, ASCA and food antigen IgG levels were correlated in both those who reported GI symptoms and those who did not. Gluten data points are shown with an '×' and a solid regression line; casein data points are shown with a closed '●' and dashed regression line. Multiple linear regressions corrected for age, sex and race were used to generate the listed p-values. NS = not significant.

leaky gut hypothesis. For example, autoimmune conditions might contribute to a heightened immune response and to inter-relatedness between the food-related antibodies, even in the absence of immune activation by specific antigens. Such a pattern may ensue due to an early developmental assault or genetic predisposition affecting immune dysfunction. Currently, there is some evidence supporting an autoimmune component of mood disorders, but results thus far are fairly mixed (63–67). Interestingly, although ASCA is predominately a marker for GI-related diseases, this marker was found to be elevated in patients with a variety of non-GI-related autoimmune conditions such as systemic lupus erythematosus, Graves' disease, cryoglobulinemia, and vasculitis (68).

Our cohort included a group of individuals with bipolar disorder who also had a recent onset of psychosis. The small sample size of this group (n = 38) limits the extent to which comparisons can be interpreted, but some interesting trends are noteworthy for future research. In contrast to our findings in the main bipolar disorder group, ASCA was correlated with antibodies to food antigens in both those who reported GI symptoms and those who did not, a possible indication of some level of nonspecificity of the immune response in the recent onset bipolar disorder group. Furthermore, ASCA levels exhibited correlations with several non-food-related antigens as well, including measles and *T. gondii*, but the positive IgG response was not uniform across all of the antigens tested. For these comparisons, however, statistical power issues might impact our sensitivity in detecting seropositivity. Of note, *T. gondii* and measles have been implicated in bipolar disorder or recent onset psychosis previously, and both have been linked, sometimes controversially, to inflammation processes that affect the GI tract in humans and in experimental animal models (6, 32, 69–82). Although our findings require further scrutiny and larger sample sizes, the possibility of a more generalized inflammatory nature inherent to earlier stages of disease cannot be discounted.

In addition to the small sample sizes of our bipolar disorder with recent onset of psychosis group, our study has a number of limitations. The study design is cross-sectional and only allows for speculation regarding mechanisms that might underlie our results. With respect to age, the recent onset psychosis group was significantly younger and the established bipolar disorder group significantly older than controls, yet both groups exhibited increased ASCA levels compared to controls, suggesting that age here may not be a strong confounding factor. Furthermore, our GI measures, the ASCA marker and self-reported GI disturbances, would benefit from an additional biological index such as a stool sample or histopathological examination of the GI tract. Blood draws were not standardized nor taken under fasting conditions, although our measures were predominately based on IgG, the levels of which are relatively stable over time and especially over the course of one day. In subanalyses, we

relied on stratifications according to most recent episode (mania, depression, or mixed), which resulted in grouping of asymptomatic with symptomatic individuals. We attempted to address this by examining correlations of ASCA with symptom severity scale scores (YMRS and HRSD) with the rationale that symptomatic individuals would score at the polar ranges of these scales. Our study revealed no correlations of ASCA with symptom severity scales. Similarly, we tried to further evaluate an effect of lifetime psychosis on ASCA levels by combining the bipolar disorder groups, but no significant differences in marker levels or intercorrelations were detected.

In conclusion, this study offers strong preliminary evidence for a role of the GI tract in the inflammatory pathology of bipolar disorder. The serological biomarkers and their inter-associations may help to identify a subgroup of individuals with bipolar disorder who may be afflicted with GI- and food-based sensitivities as well as those who may be prone to a generalized inflammatory state. Treatment strategies that involve dietary modifications, anti-inflammatory agents and/or microbiota modulations should be evaluated for effects on symptom improvement in carefully controlled clinical trials.

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