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Inflammatory Molecular Signature Associated With Infectious Agents in Psychosis

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Schizophrenia (SZ) is a devastating mental condition with onset in young adulthood. The identification of molecular biomarkers that reflect illness pathology is crucial. Recent evidence suggested immune and inflammatory cascades in conjunction with infection may play a role in the pathology. To address this question, we investigated molecular changes in cerebrospinal fluid (CSF) from antipsychotic-naïve patients with SZ and at risk mental status for psychosis (ARMS), in comparison with healthy controls (HCs). We measured 90 analytes using a broad multiplex platform focusing on immune and inflammatory cascades then selected 35 with our quality reporting criteria for further analysis. We also examined *Toxoplasma gondii* (TG) and *herpes simplex virus 1* antibody levels in CSF. We report that expression of 15 molecules was significantly altered in the patient groups (SZ and ARMS) compared with HCs. The majority of these molecular changes (alpha-2-macroglobulin [α2M], fibrinogen, interleukin-6 receptor [IL-6R], stem cell factor [SCF], transforming growth factor alpha [TGFα], tumor necrosis factor receptor 2 [TNFR2], IL-8, monocyte chemotactic protein 2 [MCP-2/CCL8], testosterone [for males], angiotensin converting enzyme [ACE], and epidermal growth factor receptor) were consistent between SZ and ARMS patients, suggesting these may represent trait changes associated with psychotic conditions in general. Interestingly, many of these analytes (α2M, fibrinogen, IL-6R, SCF, TGFα, TNFR2, IL-8, MCP-2/CCL8, and testosterone [for males]) were exacerbated in subjects with ARMS compared with subjects with SZ. Although further studies are needed, we optimistically propose that these molecules may be good candidates for predictive markers for psychosis from an early stage. Lastly, reduction of IL-6R, TGFα, and ACE was correlated with positivity of TG antibody in the CSF, suggesting possible involvement of TG infection in the pathology.

Key words: schizophrenia/at risk mental status/inflammation/cerebrospinal fluid/biomarker/*Toxoplasma gondii*

Introduction

Schizophrenia (SZ) is a mental condition with many genetic and environmental risk factors.[1] While individual genetic factors have a low effect size, environmental factors can also impact disease risk. These environmental stressors include obstetric complications, developmental nutritional deprivation, as well as possible infection with *Toxoplasma gondii* (TG) and other viruses, which are all risk factors for disease.[2–6] Furthermore, an animal model for SZ includes developmental immune activation that leads to behavioral abnormalities in the offspring.[7–9]

Recent evidence suggested that immune and inflammatory cascades in conjunction with infection may play a role in the pathology of SZ.[6,10–12] Patients with SZ displayed increased levels of TG antibodies compared with healthy controls (HCs).[13,14] Furthermore, exposure to *herpes simplex virus 1* (HSV1) was linked to poorer performance in cognitive tasks in SZ patients.[15–17] Nonetheless, the mechanism of how such environmental stressors affect the pathology of the disease is unclear.

Molecular analysis may be a useful approach to address this question. Several studies utilized the plasma or serum of SZ patients with broad multiplex screens and found changes in many molecules.[18–22] While there have been outstanding biomarker studies published using cerebrospinal fluid (CSF) from SZ patients, these studies applied candidate molecular approaches in which only a small number of molecules were tested.[23–26] As far as we are aware, broad multiplex screens have not been applied to studies using CSF from SZ patients, in particular of antipsychotic-naïve patients.

In this study, we examined expression changes in dozens of molecules in the CSF from antipsychotic-naïve patients with SZ and at risk mental status for psychosis (ARMS), compared with HCs, by utilizing a broad multiplex screen. Furthermore, we explored how disease-associated infectious agents (TG and HSV1) affect these molecular changes.

Methods

Study Participants and Sample Collection

Participants were recruited from 2 clinical sites in Germany: 46 from University of Cologne and 50 from the Central Institute of Mental Health, Mannheim. All study participants provided written informed consent, and the respective institutes' ethical committees approved the study protocol. Procedures were performed in accordance with the code of ethics of the world medical association for experiments involving humans (Declaration of Helsinki). HCs had no history of mental illness or psychotic episodes and were recruited within the same geographic area and population. Patients defined as having SZ met criteria of the DSM-IV. ARMS patients met the following 3 alternative criteria: (1) attenuated positive symptoms, (2) brief limited intermittent psychotic symptoms that spontaneously resolved within 1 week, and (3) a recent decline in function for at least 1 month, in combination with the existence of a first or second degree relative with a history of any DSM-IV psychotic disorder, or met criteria for schizotypal personality disorder.[27–29] All patients were antipsychotic naïve, but some ARMS patients received low-dose benzodiazepines. Demographic information for age, gender, body mass index (BMI), ethnicity, years of education, and smoking status is given in table 1. Patients and HCs were demographically similar for age, gender, BMI, and smoking status as described in Methods and table 1. Demographic information for each cohort is provided in supplementary table 1. CSF samples were collected and stored at −80°C or lower, and samples were thawed for aliquoting before each analysis.[5]

Analyte Measurements

Sample aliquots were shipped to Myriad-Rules Based Medicine (RBM) for evaluation using the multianalyte profiling based on multiplex immunoassay platforms to simultaneously quantify panels of analytes. Sample processing at RBM facilities were described in detail previously.[26] We designed a custom analyte panel of 90 markers including inflammatory cytokines, chemokines, metabolic factors, and growth factors. Assays were developed, validated, and raw intensities converted to absolute concentrations by comparison with a standard curve by the Clinical Laboratory Improvement Amendment certified laboratories at Myriad-RBM. Final data were reported as the absolute concentrations in the CSF, less than the least detectable dose (LDD), or quantity not sufficient (QNS).

Infectious Antibody Measurements

TG immunoglobulin G (IgG) antibody levels were measured using a commercially available ELISA kit from IBL America. HSV1 IgG antibody levels were measured using a commercially available ELISA kit from Focus Diagnostics. All assays were performed according to the manufacture instructions. The IgG levels for these infectious agents were recorded as absorbance values and LOG normalized before further analysis. Kit standards were run with each plate to control for interplate variation and identify antibody positive and negative individuals.

Statistical Analysis

All statistical analyses were performed using the R statistical analysis software (version 3.0.2). Reproducible R code for each analysis is provided in the supplementary methods.

Patients and HCs Demographic Characteristics. HCs and patients with SZ, and ARMS across both cohorts (Cologne and Mannheim) were comparable for age ($P = 0.53$) and BMI ($P = 0.91$) using analysis of variance (ANOVA) across each diagnosis (table 1). Demographic similarity for gender (female or male, $P = 0.088$), education duration (≥13 years or <13 years, $P = 1.04E{-}08$), and smoking (yes or no, $P = 0.074$) was analyzed using a 2×3 Fisher's exact test (table 1). Ethnicity (Caucasian, Asian, or Other, $P = 0.040$) was analyzed using a 3×3 Fisher's exact test (table 1). The patients completed significantly fewer years of education than the HCs possibly due to a need for treatment during the illness. Ethnicity was not statistically similar, but the vast majority of patients were Caucasian with only 3 non-Caucasian individuals, 1 HC and 2 patients (table 1).

Analyte Expression Analysis. Among the 90 analytes we examined, we specifically selected 35 for further analysis based on having less than 5% QNS values and at least 75% of values reported: 77% (27 analytes) required no imputing, 11% (4 analytes) required less than 15% imputation, and 11% (the last 4 analytes) required more than 15% imputation (supplementary table 2). All data were LOG normalized before further analysis. We fit a parametric Gaussian survival regression model relating each analyte to diagnosis, cohort, and smoking status. Removing smoking status from our model did not affect the result outcomes; thus, smoking status does not significantly contribute to the results (see supplementary methods for R code). The survival model treated the analytes as left censored if the values were below the

Table 1. Clinical and Demographic Information for Patients and HCs

	HCs	SZ	ARMS	Test	*P* Value
N	35	46	15		
Age[a]	26.4 ± 0.6	25.8 ± 0.8	24.9 ± 1.0	ANOVA	0.531
Gender (female/male)	14/21	10/36	7/8	Fisher's Exact	0.088
BMI[a]	23.3 ± 0.5	23.0 ± 0.4	23.1 ± 1.1	ANOVA	0.912
Ethnicity[b]	34/1/0	46/0/0	13/1/1	Fisher's Exact	0.040
Education[c] (≥13/<13 years)	33/1	21/25	4/11	Fisher's Exact	1.04E−08
Smoking (yes/no)	17/18	30/16	5/10	Fisher's Exact	0.074
Antipsychotic medication	0%	0%	0%		

Note: Demographic information is reported for all participants within each diagnostic group (healthy controls [HCs], schizophrenia [SZ], and at risk mental status for psychosis [ARMS]). We report information on the number of participants (*N*), participants' age, gender, body mass index (BMI), ethnicity, years of education (percentage that completed at least 13 years, a high-school diploma equivalent), smoking, and antipsychotic treatment (all patients were antipsychotic naïve). HCs and patients were statistically similar for age, gender, BMI, and smoking. Significantly fewer patients finished their education during illness. The majority of participants were Caucasian, but 3 individuals were non-Caucasian, 1 HC and 2 patients.
[a]Mean ± SEM.
[b]Caucasian/Asian/Other.
[c]Individuals who completed at least 13 years of education or a high-school diploma equivalent.

LDD. The model was fit using the survival package in R, see supplementary material R code and documentation for exact details of the model.[30–32] The QNS values were not included in the analysis. An ANOVA (lambda = 0.7) among the diagnoses was performed on the survival model. For testosterone, the females were below the LDD; thus, the analyses were computed on only the male participants. Fold change was calculated as the ratio of the median expression of patients to HCs. Correction for multiple comparisons was evaluated using a *q*-value conversion to control the false discovery rate; thus, a *q* value below 0.05 is considered significant.[33] Median was calculated from the untransformed data and reported as median and interquartile range (IQR). Box and whisker plots were used to graphically represent the transformed data providing the median, first and third quartile, range, and outliers. We performed a correlation between each analyte to identify related analytes and represented the data in a heatmap with the strongest correlations listed in a table; missing data were imputed to the LDD or the analyte average for values below the LDD and QNS, respectively (see supplementary methods for R code and supplementary figure 1).

Comparison of Infectious Agents in Patients vs HCs. To compare the level of infectious agents between each diagnostic group, we performed an ANOVA for the level of infectious antibodies (HSV1 or TG) among the diagnoses (HCs and patients with SZ and ARMS) using site as a covariate. Next, to further investigate significant associations, we performed a Fisher's exact test by assigning the participants into dichotomous positive or negative groups for HSV1 and TG based on the negative control values in each ELISA assay.

Infectious Agent and Analyte Correlation Analysis. Similar to above, parametric survival analysis was performed on normalized data, and an ANOVA was performed between the infectious agents (HSV1 and TG) and each analyte. Correction for multiple comparisons was performed using *q* value conversion.[33] In addition, a linear regression was performed on the significant analytes (TG $q < 0.05$, HSV1 $p < 0.05$), and the correlation coefficient (*r*) and adjusted R^2 are reported. Next, the participants were categorized as HSV1 positive, HSV1 negative, TG positive, and TG negative depending on whether the antibody level was above or below the negative control, and a Student's *t*-test was performed on the analyte expression level of the identified molecules between those positive and negative for each infectious agent.

Results

Differential Molecular Expression Between Patients With Psychosis and HCs

First, we compared the HC group with the patient group (SZ and ARMS together), which resulted in 5 differentially expressed analytes (table 2, supplementary figures 2–3): Angiotensin converting enzyme (ACE) and fibrinogen were significantly reduced in the patient group (supplementary figure S2A-B), whereas interleukin-8 (IL-8), monocyte chemotactic protein 2 (MCP-2/CCL8), and testosterone were increased (supplementary figure S2C-E). Testosterone was compared only in male participants.

Next, we compared HC, SZ, and ARMS groups separately. Fifteen analytes were significantly different among these groups (table 3). Depending on the pattern of expression change, we divided these 15 analytes into 5 subgroups (figure 1, supplementary figure 4). Six analytes in group 1A (alpha-2-macroglobulin [α2M], fibrinogen,

Table 2. Differential Analyte Expression Between all Patients and HCs

Protein	Units	HC[a]	Psy[a]	Fold Change	*q* Value
ACE	ng/ml	1.60 (0.75)	1.30 (0.50)	0.81	0.035
Fibrinogen	μg/ml	0.170 (0.14)	0.120 (0.16)	0.71	0.011
IL-8	pg/ml	21.0 (9.5)	27.0 (14)	1.29	0.011
MCP-2/CCL8	pg/ml	2.15 (0.50)	3.10 (1.5)	1.44	0.011
Testosterone	ng/ml	0.085 (0.088)	0.185 (0.11)	2.18	0.011

Note: Analyte expression from all patients (Psy) compared with healthy controls (HCs). Angiotensin converting enzyme (ACE) and fibrinogen were decreased in the patient population, whereas interleukin-8 (IL-8), monocyte chemoattractant protein 2 (MCP-2/CCL8), and testosterone were increased in the patient population. Testosterone was only compared in male participants. IQR = interquartile range, Fold change = Psy/HCs.
[a]Median (IQR).

Table 3. Differential Analyte Expression Among HCs and SZ and ARMS Patients

Analyte	Units	HCs[a]	SZ[a]	ARMS[a]	FC, SZ/ARMS	*q* Value	Group
α2M	μg/ml	3.30 (0.80)	3.05 (0.80)	2.80 (0.80)	0.92/0.85	0.023	1A
ACE	**ng/ml**	**1.60 (0.75)**	**1.35 (0.50)**	**1.30 (0.71)**	**0.84/0.81**	**0.020**	**2**
Adiponectin	ng/ml	3.60 (2.25)	3.30 (2.2)	1.50 (2.1)	0.92/0.42	0.026	3
C3	μg/ml	1.90 (1.10)	2.00 (0.88)	1.70 (0.7)	1.05/0.89	0.037	4
EGFR	ng/ml	0.140 (0.04)	0.125 (0.052)	0.120 (0.058)	0.89/0.86	0.027	2
Fibrinogen	**μg/ml**	**0.170 (0.14)**	**0.130 (0.22)**	**0.064 (0.13)**	**0.76/0.38**	**0.020**	**1A**
IL-6	pg/ml	0.920 (0.58)	1.400 (1.07)	0.690 (0.77)	1.52/0.75	0.020	4
IL-6R	ng/ml	0.970 (0.43)	0.855 (0.34)	0.750 (0.39)	0.88/0.77	0.023	1A
IL-8	**pg/ml**	**21.00 (9.5)**	**25.00 (15.0)**	**27.00 (9.0)**	**1.19/1.29**	**0.022**	**1B**
MMP3	ng/ml	0.160 (0.09)	0.155 (0.088)	0.110 (0.059)	0.97/0.69	0.045	3
MCP-2/CCL8	**pg/ml**	**2.15 (0.50)**	**2.90 (1.65)**	**3.40 (0.95)**	**1.35/1.58**	**0.020**	**1B**
SCF	pg/ml	49.00 (17.5)	44.00 (17.5)	38.00 (18.5)	0.90/0.78	0.026	1A
TGFα	pg/ml	22.00 (7.0)	20.00 (9.0)	19.00 (0.15)	0.91/0.86	0.020	1A
TNFR2	ng/ml	0.490 (0.16)	0.440 (0.23)	0.380 (0.26)	0.90/0.78	0.041	1A
Testosterone	**ng/ml**	**0.085 (0.088)**	**0.150 (0.10)**	**0.235 (0.068)**	**1.76/2.76**	**0.020**	**1B**

Note: Expression changes between healthy controls (HCs), schizophrenia (SZ), and at risk mental status for psychosis (ARMS) were the following: Alpha-2 macroglobulin (α2M), angiotensin converting enzyme (ACE), complement 3 (C3), epidermal growth factor receptor (EGFR), fibrinogen, interleukin-6 (IL-6), interleukin-6 receptor (IL-6R), interleukin-8 (IL-8), matrix metalloproteinase 3 (MMP3), monocyte chemoattractant protein 2 (MCP-2/CCL8), stem cell factor (SCF), transforming growth factor alpha (TGFα), tumor necrosis factor receptor 2 (TNFR2), and testosterone. Fold change (FC) reported as (SZ/HCs)/(ARMS/HCs). Bold values were listed in table 2. Testosterone analyzed only in males. IQR = interquartile range.
[a]Median (IQR).

interleukin-6 receptor [IL-6R], stem cell factor [SCF], transforming growth factor alpha [TGFα], and tumor necrosis factor receptor 2 [TNFR2]) were decreased in the SZ group with further reduction in the ARMS group (figures 1A–F). Three analytes in Group 1B (IL-8, MCP-2/CCL8, and testosterone [males]) were increased in the SZ group with further augmentation in the ARMS group (figures 1G–I). We speculate that these 2 groups represent molecular changes associated with psychosis, but the extent of the changes (regardless of increase or decrease) is greater in the risk stage. Group 2 analytes (ACE and epidermal growth factor receptor [EGFR]) were decreased in patients with SZ and ARMS similarly, possibly representing traits associated with psychosis in general (figures 1J and K). In contrast, group 3 analytes (adiponectin and matrix metalloproteinase 3 [MMP3]) were uniquely decreased only in ARMS patients (figures 1L and M). Finally, group 4 (complement 3 [C3] and interleukin-6 [IL-6]) showed an increase in SZ patients and decrease in subjects with ARMS (figures 1N and O): C3 was only slightly increased in SZ patients with a more prominent decrease in the ARMS group, whereas IL-6 showed a substantial increase in SZ patients and a slight decrease in patients with ARMS.

Infectious Agents Effect on Analyte Expression

Given that psychotic disorders, such as SZ, occur as the outcome of gene and environmental interactions, it is very important to clarify how environmental stressors, such as infectious agents, may be related to the molecular signatures we uncovered above. To address this question,

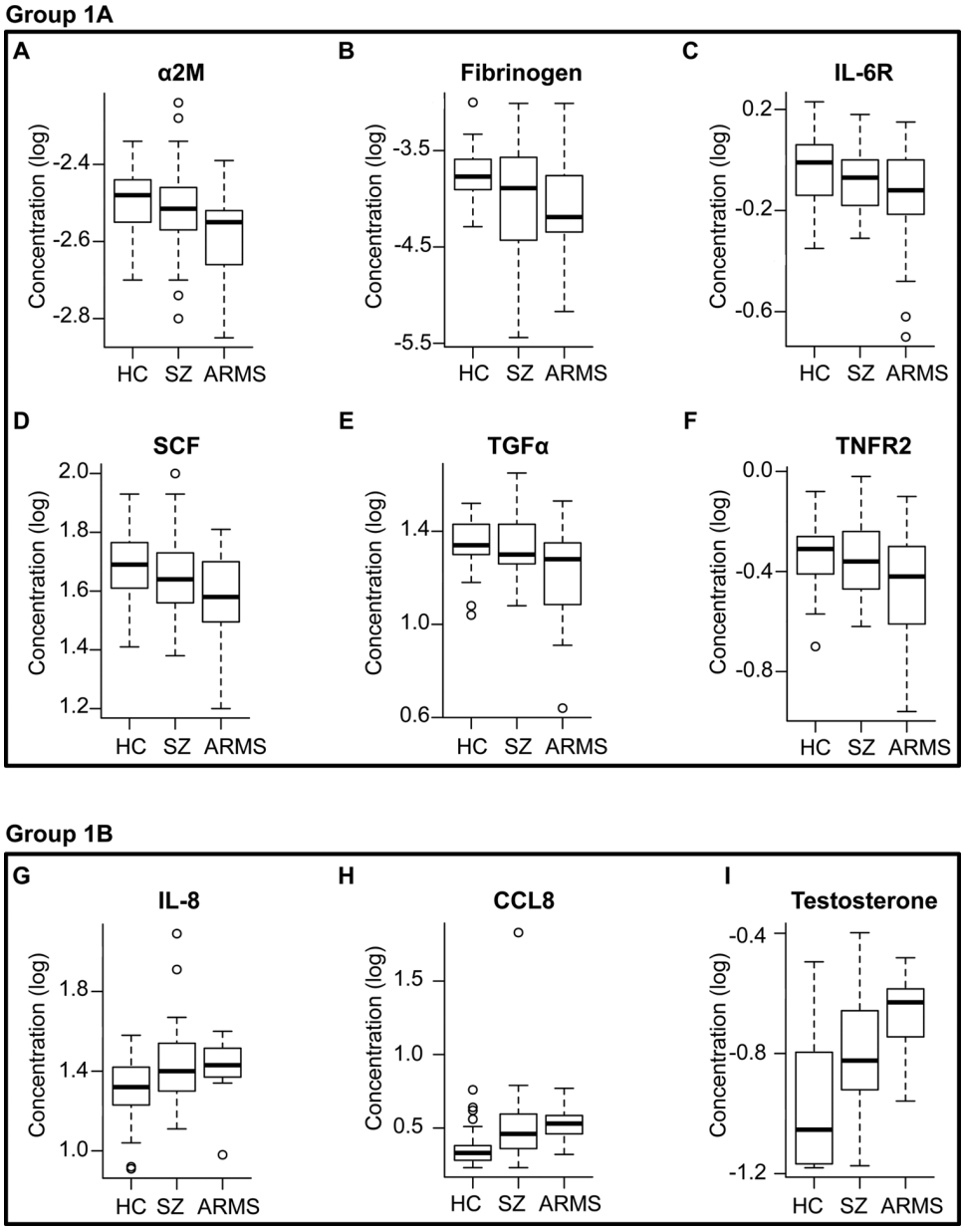

Fig. 1. Box and whisker plots of differentially expressed analytes between patients with schizophrenia (SZ), at risk mental status for psychosis (ARMS), and healthy controls (HCs). (A–F) (Group 1A) Analytes with decreased expression in SZ patients with ARMS showing the greatest decrease. Group 1A included α-2 macroglobulin (α2M), fibrinogen, interleukin-6 receptor (IL-6R), stem cell factor (SCF), transforming growth factor α (TGFα), and tumor necrosis factor receptor 2 (TNFR2). (G–I) (Group 1B) Analytes with increased expression in SZ patients and further increase in ARMS patients, including IL-8, monocyte chemotactic protein 2 (MCP-2/CCL8), and testosterone. (J and K) (Group 2) Angiotensin converting enzyme (ACE) and epidermal growth factor receptor (EGFR) were decreased comparably in SZ and ARMS patients. (L and M) (Group 3) Adiponectin and matrix metalloproteinase 3 (MMP3) were only decreased in ARMS patients. (N and O) (Group 4) Complement 3 (C3) and IL-6 in SZ and ARMS patients were changed in opposite directions. C3 showed a slight increase in SZ patients with a more pronounced decrease in ARMS patients. IL-6 showed a substantial increase in SZ patients and a slight decrease in ARMS patients. Testosterone was only analyzed in males. The analyte concentrations were LOG transformed before analysis; thus, the axis displays the transformed concentration (see table 3 for values). Box and whisker plots display the median (bold line), first and third quartile (box), range (dashed lines), and outliers (circles).

we measured the level of antibodies for HSV1 and TG in the CSF and correlated the antibody levels with the changes in analyte expression studied above.

First, we failed to observe a significant change in the antibody levels between groups of HC and patients (SZ and ARMS together) for HSV1 or TG. Thus, we next addressed the correlation between each analyte expression and antibody level evaluating all groups together by an unbiased parametric survival analysis. As for HSV1, the antibody levels were correlated with α2M, ACE, TGFα,

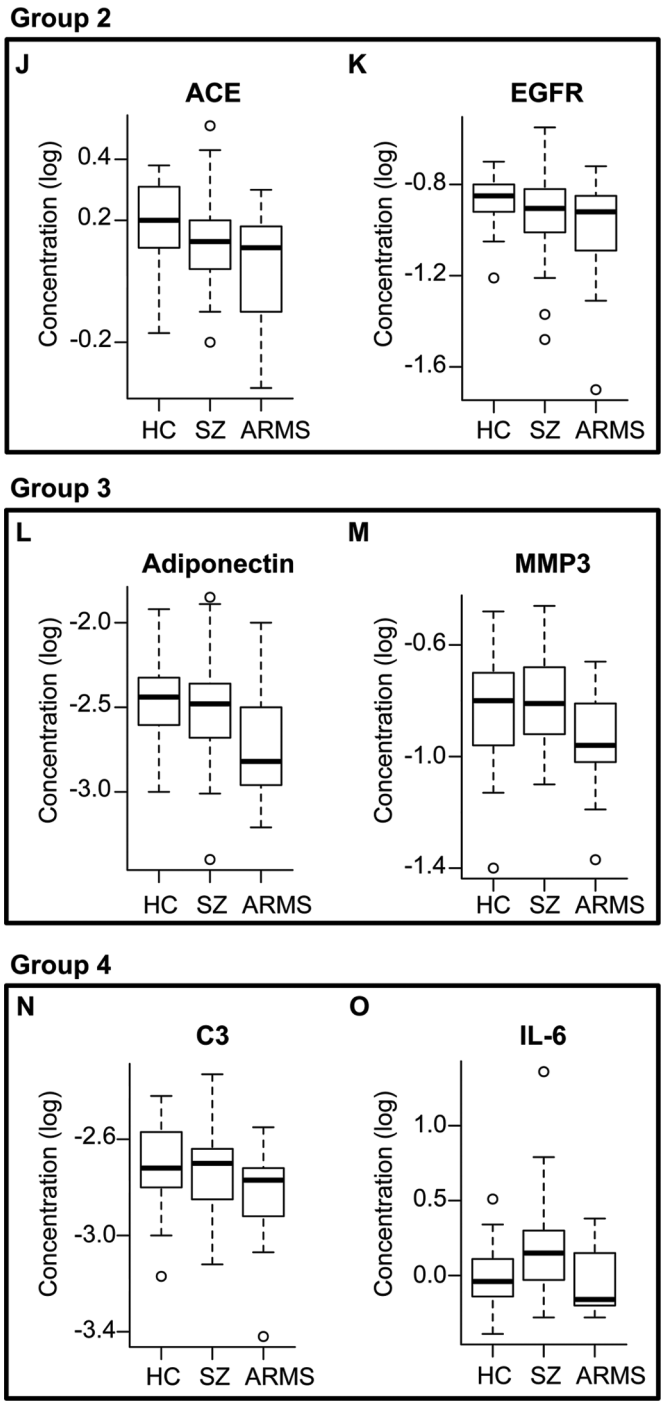

Fig. 1. Continued

and vitamin D binding protein (VDBP) (table 4A). However, these did not remain significant after multiple comparison correction. In contrast, the levels of an anti-TG antibody were significantly correlated with α-1 anti-trypsin (AAT), ACE, β-2 microglobulin (β2M), EGFR, heparin binding-epidermal growth factor (HB-EGF), IL-6R, macrophage inflammatory protein β (CCL4), SCF, and TGFα, all of which survive multiple comparison correction and remain statistically significant (table 4A).

Next, we divided the subjects into 4 groups: HSV1 positive, HSV1 negative, TG positive, and TG negative (as described in the Methods section, the threshold was determined by the kit standards). Then we tested whether the protein expression, listed in table 4A, was different between these positive and negative groups. We found that the levels of α2M, ACE, TGFα, and VDBP were significantly higher in the CSF of HSV1-positive subjects compared with HSV1-negative subjects (table 4B).

Table 4. Correlation of Analyte Expression With Each Infectious Agent

(A)

HSV1	*r*	R^2	*P* Value	*q* Value
α2M*	1.1414	0.0485	0.0161	0.0871
ACE*	0.6908	0.0371	0.0312	0.0871
TGFα*	0.7246	0.0352	0.0348	0.0871
VDBP	0.4227	0.0389	0.0281	0.0871
TG				
AAT	−0.5461	0.0343	0.0367	0.0329
ACE*	−0.6836	0.0429	0.0222	0.0329
β2M	−0.4763	0.0282	0.0524	0.0329
EGFR*	−0.7412	0.0630	0.0070	0.0329
HB-EGF	−2.0272	0.0498	0.0149	0.0329
IL-6R*	−0.6377	0.0352	0.0348	0.0329
MIP-1β/CCL4	−0.4779	0.0336	0.0382	0.0329
SCF*	−0.6802	0.0330	0.0395	0.0329
TGFα*	−0.6816	0.0358	0.0335	0.0329

(B)

HSV1	Positive[a], *N* = 34	Negative[a], *N* = 62	*P* Value
α2M (μg/ml)	3.42 ± 0.15	2.96 ± 0.08	**0.016**
ACE (ng/ml)	1.67 ± 0.10	1.40 ± 0.06	**0.019**
TGFα (pg/ml)	24.12 ± 1.22	20.62 ± 0.78	**0.016**
VDBP (μg/ml)	1.34 ± 0.11	1.08 ± 0.08	**0.023**
TG	Positive[a], *N* = 18	Negative[a], *N* = 78	*P* Value
AAT (μg/ml)	5.08 ± 0.57	6.19 ± 0.22	0.064
ACE (ng/ml)	1.26 ± 0.13	1.55 ± 0.06	**0.043**
β2M (μg/ml)	0.666 ± 0.06	0.851 ± 0.047	0.055
EGFR (ng/ml)	0.112 ± 0.01	0.135 ± 0.005	0.072
HB-EGF (pg/ml)	152.4 ± 4.96	164.2 ± 2.35	**0.049**
IL-6R (ng/ml)	0.74 ± 0.06	0.92 ± 0.03	**0.040**
MIP-1β/CCL4 (pg/ml)	16.6 ± 1.40	21.5 ± 1.32	0.067
SCF (pg/ml)	41.2 ± 3.60	48.8 ± 1.80	0.067
TGFα (pg/ml)	18.7 ± 1.38	22.6 ± 0.75	**0.040**

Note: (A) Correlation of each infectious agent antibody levels with the CSF analyte expressions. The top correlations are listed. HSV1 correlations with α2M, ACE, TGFα, and vitamin D binding protein (VDBP) had a $P < 0.05$ but did not maintain significance after multiple comparison correction ($q > 0.05$). TG was significantly correlated with α-1 antitrypsin (AAT), ACE, β-2-microglobulin (β2M), EGFR, heparin binding epidermal growth factor (HB-EGF), IL-6R, macrophage inflammatory protein 1β (MIP-1β/CCL4), SCF, and TGFα ($q < 0.05$). *r* = correlation coefficient. R^2 = adjusted R^2. Asterisks indicate analytes differentially expressed in patients from table 3. (B) HSV1-positive participants expressed significantly more α2M, ACE, TGFα, and VDBP. TG-positive participants expressed significantly less ACE, HB-EGF, IL-6R, and TGFα. Analyte expression is reported as mean ± standard error of the mean (SEM). Bold values indicate significance. *N* = number of participants in each group.
[a]Mean ± SEM.

Interestingly, for the TG positive subjects, we found significantly less expression of ACE, HB-EGF, IL-6R, and TGFα (table 4B).

Discussion

In this study, we reported 15 analytes that were differentially expressed among HC, SZ, and ARMS subjects. These analytes were subdivided into 5 groups, depending on the pattern of expression change. We highlighted sets of analytes that are changed consistently in SZ and ARMS patients compared with HCs. Moreover, the extent of change in some analytes was greater in the ARMS group compared with the SZ group. Furthermore, we showed how the change of these molecules is related to disease-associated infectious agents, HSV1 and TG.

We believe this study has strengths in the following 2 points. First, all subjects with SZ and ARMS were antipsychotic naïve and only a small number of subjects received low doses of benzodiazepine. Thus, this

sample set is very useful to address the molecular signature directly associated with psychotic disorders, particularly in the early phase of psychosis. Furthermore, in contrast to most studies with CSF that utilized candidate molecular approaches in which only a small number of target molecules were evaluated per study, we used a broad multiplex platform to test dozens of molecules simultaneously. Second, in addition to addressing molecular changes in patients with psychosis, we explored the possible relationship of such changes in the CSF to disease-associated infectious agents by noting the CSF antibody levels against HSV1 and TG.

Our study highlighted important molecular markers for psychosis but was limited by our small sample size and difference in educational level. The difference in education maybe due to disrupted education or cognitive dysfunction in patients but not in HCs. To accurately represent the HCs and avoid introducing a bias, we did not restrict our HCs to a less educated population. However, the patients and controls were similar for the other demographic characteristics.

Some studies, but not all, demonstrated an increased level of TG antibodies in patients with SZ. Our results do not negate these findings but merely were not replicated in our subset of participants. We further analyzed how infectious agents might affect the CSF analytes beyond the classification of diagnosis, based on the idea that the infectious agents affect host factors that underlie the pathophysiology of the cases. Our findings are further validated because previous studies found that HSV1 binding to α2M promoted infectivity in a neuronal cell line.[34] In addition, a previous study showed infection of macrophages, *in vitro*, with TG blocked production of proinflammatory cytokines, such as IL-6, and blocked the increase of over 50 LPS-induced cytokines,[35] providing evidence for broad protein suppression upon TG infection.

A number of the differentially expressed proteins uncovered in this study directly impact EGF signaling. Overall, we found decreases in EGFR, fibrinogen, and TGFα in patients compared with HCs. We also found a negative correlation of TG with EGFR, HB-EGF, and TGFα. EGFR (ErbB1) is also relevant in SZ because EGFR forms heterodimers with other ErbB receptors, such as ErbB4, a known genetic risk factor for SZ[36]. Thus, EGF signaling may be a candidate to investigate gene and environmental interactions for psychotic conditions. Furthermore, previous studies of cardiovascular and renal disease identified that ACE can affect the activation of EGF signaling by modulating the phosphorylation status of the EGF receptors or the downstream EGFR signaling intermediates.[37,38]

It is also interesting that the IL-6 ligand and receptor were significantly changed in opposite directions in SZ patients. Coughlin et al[26] also reported this observation of IL-6/IL-6R in the CSF from recent-onset SZ patients. Conceivably, an increase in the level of IL-6 ligand in the context of immune activation drives a subsequent decrease in IL-6R as a means of compensating for the overproduced ligand. In addition, the idea of compensation is observed by the increase in testosterone and decrease in ACE. A human study of multiple sclerosis showed treatment with testosterone provided immune protection and symptom improvement.[39] Furthermore, ACE produces angiotensin II that binds to the angiotensin receptor 1 to elicit a proinflammatory immune response in microglia.[40] Taken together, increased testosterone, decreased ACE, and increased IL-6R may be comprehensively interpreted as homeostatic compensation for a baseline increase in inflammation in patients.

It is noteworthy that the majority of the molecular changes (11 out of 15, α2M, fibrinogen, IL-6R, SCF, TGFα, TNFR2, IL-8, MCP-2/CCL8, testosterone [for males], ACE, and EGFR] were consistent between SZ and ARMS groups compared with HCs. Thus, these likely represent trait changes associated with psychotic conditions in general. Among these, disease-associated reduction of IL-6R, TGFα, and ACE was correlated with positivity of TG antibody.

Identifying early molecular changes is important for early detection and treatment often leading to better outcomes. Of clinical interest in this study, the ARMS subjects exhibited greater changes in most of these analytes (9 out of 11) compared with the SZ patients. Given that ARMS included subjects at risk for or in early stage of psychosis and SZ, the intermediate molecular profile observed in SZ may be the result of compensatory and homeostatic feedback mechanisms initiated after the early stage. There is precedence that disease signatures become less prominent during the course of disease progression as some viral infections, such as human immunodeficiency virus (HIV) or syphilis, can have dormant phases after a more severe initial infection. Although further studies with much larger samples are needed, we optimistically suggest that these 9 molecules (α2M, fibrinogen, IL-6R, SCF, TGFα, TNFR2, IL-8, MCP-2/CCL8, and testosterone [for males]) may be good candidates for predictive markers for psychosis from an early stage.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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