

Coronavirus Immunoreactivity in Individuals With a Recent Onset of Psychotic Symptoms

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Prenatal influenza exposure increases the risk for schizophrenia and brings to question how other respiratory viruses may contribute to neuropsychiatric disease etiopathology. Human coronaviruses cause respiratory infections that range in seriousness from common colds to severe acute respiratory syndrome. Like influenza, coronaviruses can be neurotropic. To test for associations between coronaviruses and serious mental disorders, we utilized a recently developed assay and measured immunoglobulin G (IgG) response against 4 human coronavirus strains (229E, HKU1, NL63, and OC43) in 106 patients with a recent onset of psychotic symptoms and 196 nonpsychiatric controls. We expressed results quantitatively as antibody levels and qualitatively as seroprevalence relative to a defined seropositivity cutoff value. Patient IgG levels were higher than controls for HKU1, NL63, and OC43, with HKU1 and NL63 both showing highly significant patient-to-control differences (HKU1, $P \leq .002$; NL63, $P \leq .00001$). All 4 coronaviruses were more seroprevalent in patients vs controls, with greatest intergroup differences observed for HKU1 (93% vs 77%, $P \leq .0001$). HKU1 and NL63 associations with the patient group were further supported by multivariate analyses that controlled for age, gender, race, socioeconomic status, and smoking status (HKU1, odds ratio [OR] = 1.32, 95% confidence interval [CI] = 1.03–1.67, $P \leq .027$; NL63, OR = 2.42, 95% CI = 1.25–4.66, $P \leq .008$). Among patients, NL63 was associated with schizophrenia-spectrum (OR = 3.10, 95% CI = 1.27–7.58, $P \leq .013$) but not mood disorders. HKU1 and NL63 coronavirus exposures may represent comorbid risk factors in neuropsychiatric disease. Future studies

should explore links between the timing of coronavirus infections and subsequent development of schizophrenia and other disorders with psychotic symptoms.

Key words: schizophrenia/infection/immunology/pathogen/bipolar disorder/virus

Introduction

Prenatal and perinatal infections are associated with the onset of adult psychiatric illness in some susceptible individuals.^{1,2} Maternal exposure to *Toxoplasma gondii*, influenza, measles, polio, and genital and/or reproductive infections confers an increased risk of schizophrenia to the developing offspring.^{3–10} Childhood infections such as bacterial or viral meningitis may also play a role in psychotic disease etiology.^{11,12} The connection between adult infections and schizophrenia is less clear-cut.² Serological collections that include samples taken prior to disease diagnosis can provide valuable information regarding microbial exposure at the time of symptom onset in adult populations. In a prospective study of a US military cohort, antibodies to *T. gondii* and human herpesvirus 6 were significantly associated with the subsequent development of schizophrenia in some individuals.^{13,14}

Respiratory viruses such as influenza viruses and coronaviruses are potentially neurotropic and can enter the brain via the olfactory neural pathway.^{15–18} Human coronaviruses cause infections ranging from common colds to severe acute respiratory syndrome (SARS).^{19,20} Coronaviruses are single-stranded RNA viruses with outer envelopes that have distinct crown-like morphologies. Non-SARS respiratory infections occur from group I (229E and NL63) and group II (OC43 and HKU1) coronaviruses. 229E and OC43 were first described in the 1960s,^{21–23} whereas NL63 and HKU1 were more recently discovered and first described in 2004–2005.^{24,25} Data from clinical, postmortem, in vitro, and animal studies support that coronavirus exposure can have neurological consequences including psychiatric symptoms and encephalitis.^{26–33} Clinical reports of psychiatric symptoms such as auditory and visual hallucinations and manic and depression disorders have been described in studies

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of SARS infection.^{28,32} Coronavirus RNA has been found in human brain autopsy samples of individuals with multiple sclerosis and in those with SARS.^{26,29,33} Because these viruses are known to infect neurons, have been associated with neuropsychiatric effects in SARS, and are not antigen targets of currently administered vaccines, they are good candidates for studies of the role of adult infections in neuropsychiatric illnesses.

We have previously described the development of serological assays specific for the immunodominant nucleocapsid protein of each non-SARS human coronavirus (229E, HKU1, NL63, and OC43) and a feline coronavirus that is not known to cause infections in humans.³⁴ We have also previously described a unique study population that is composed of a group of patients who have experienced the recent onset of psychotic symptoms and are subsequently diagnosed with a specific neuropsychiatric disease.³⁵ Here, we compared coronavirus immunoglobulin G (IgG) antibody levels in this group with those from healthy, nonpsychiatric adults to determine the extent that coronavirus exposure may correlate with the recent onset of serious mental illness.

Methods

Study Participants

We recruited 106 individuals with a recent onset of psychotic symptoms by screening consecutive admissions to inpatient and day hospital programs of the Sheppard Pratt Health System, a large not-for-profit psychiatric center in Baltimore, MD. Details of the screening population have been previously described.³⁵ Inclusion criteria 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 occurred several times a week; age between 18 and 45 years, inclusive; and voluntary admission to either the inpatient or day hospital program. Exclusion criteria were mental retardation; psychotic symptoms which occurred only in the context of substance abuse, intoxication, or withdrawal; history of intravenous drug use; and general medical conditions such as human immunodeficiency virus (HIV) or seizure disorders that might affect cognitive status. Half of the individuals with a recent onset of psychotic symptoms were diagnosed with mood disorders ($n = 53$) and the other half with schizophrenia and other psychotic disorders ($n = 53$), based on criteria defined by *Diagnostic and Statistical Manual Mental Disorders* (Fourth Edition, Text Revision) (*DSM-IV-TR*) Axis I disorders.³⁶ Specific diagnoses, *DSM-IV-TR* codes, and sample sizes are listed in table 1.

A total of 196 individuals without a history of psychiatric disorder were recruited from posted announcements and were screened to rule out current or past psychiatric disorders with the Structured Clinical Interview for *DSM-IV* Axis I disorders.³⁷ Participants were between

Table 1. Subsequent Diagnoses of Individuals With a Recent Onset of Psychotic Symptoms

Code	Diagnosis	<i>n</i>	%
Schizophrenia-spectrum disorders		53	
295.3	Schizophrenia, paranoid type	7	13.2
295.4	Schizophreniform disorder	19	35.8
295.7	Schizoaffective disorder	8	15.1
295.9	Schizophrenia, undifferentiated type	6	11.3
297.1	Delusional disorder	2	3.8
298.8	Brief psychotic disorder	2	3.8
298.9	Psychotic disorder not otherwise specified	9	17.0
Mood disorders		53	
296.04	Bipolar I disorder, single manic episode, severe with psychotic features	2	3.8
296.24	Major depressive disorder, single episode, severe with psychotic features	4	7.5
296.34	Major depressive disorder, recurrent, severe with psychotic features	12	22.6
296.44	Bipolar I disorder, most recent episode manic, severe with psychotic features	16	30.2
296.53	Bipolar I disorder, most recent episode depressed, severe without psychotic features	1	1.9
296.54	Bipolar I disorder, most recent episode depressed, severe with psychotic features	7	13.2
296.64	Bipolar I disorder, most recent episode mixed, severe with psychotic features	9	17.0
296.89	Bipolar II disorder	2	3.8

the ages of 18 and 65 years, inclusive, and had none of the following: current substance abuse over the past 1 month or any history of intravenous substance abuse; mental retardation; medical disorder that would affect cognitive performance such as epilepsy, history of encephalitis or head trauma, or any other reported neurological disorder of the central nervous system; or clinically apparent herpesvirus infection or recent treatment with antiviral medications.

Basic demographic data of the study populations are shown in table 2. Diagnostic groups differed significantly in age, gender, maternal education levels, and smoking status. These variables were included in the multivariate analyses described below.

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

The studies were approved by the Institutional Review Board of the Sheppard Pratt Health System and the Johns Hopkins Medical Institution following established guidelines. This investigation was carried out in accordance with the latest version of the Declaration of Helsinki. All participants provided written informed consent after study procedures were explained.

Table 2. Demographics of the Study Subjects

	<i>n</i>	Age, Mean Years ± SEM	African American, <i>n</i> (%)	Caucasian, <i>n</i> (%)	Other Race, <i>n</i> (%)	Males, <i>n</i> (%)	Females, <i>n</i> (%)	Maternal Education, Mean Years ± SEM	Smokers, <i>n</i> (%)	Nonsmokers, <i>n</i> (%)
Controls ^a	196	34.16 ± 0.84	56 (28.6)	129 (65.8)	11 (5.6)	71 (36.2)	125 (63.8)	13.31 ± 0.22	44 (22.4)	152 (77.6)
Recent onset	106	24.61 ± 0.78 ^b	32 (30.2)	66 (62.3)	8 (7.5)	59 (55.7) ^c	47 (44.3)	14.18 ± 0.26 ^d	39 (36.8) ^e	67 (63.2)
Mood disorders	53	25.78 ± 1.14 ^b	13 (24.5)	35 (66.1)	5 (9.4)	20 (37.7)	33 (62.3)	14.14 ± 0.37	19 (35.8) ^e	34 (64.2)
Schizophrenia-spectrum disorders	53	23.44 ± 1.03 ^b	19 (35.8)	31 (58.5)	3 (5.7)	39 (73.6) ^c	14 (26.4)	14.22 ± 0.38	20 (37.7) ^e	33 (62.3)

^aAll statistical tests compare the patient group to the control group.

^bRecent onset: $t = -7.5$, $P \leq .0001$; mood disorders: $t = 4.9$, $P \leq .0001$; schizophrenia-spectrum disorders: $t = 6.3$, $P \leq .0001$.

^cRecent onset: $\chi^2 = 10.6$, $P \leq .001$; schizophrenia-spectrum disorders: $\chi^2 = 23.6$, $P \leq .001$.

^dRecent onset ($n = 101$): $t = 2.4$, $P \leq .02$.

^eRecent onset: $\chi^2 = 7.1$, $P \leq .008$; mood disorders: $\chi^2 = 4.0$, $P \leq .046$; schizophrenia-spectrum disorders: $\chi^2 = 5.1$, $P \leq .024$.

Coronavirus Assay Development and Validation

Development and application of the coronavirus assay has been previously described.³⁴ In brief, recombinant glutathione s-transferase (GST)-fusion nucleocapsid proteins for human coronaviruses 229E, HKU1, NL63, and OC43 and a feline coronavirus were generated via baculovirus cloning, and proteins were expressed in *Trichoplusia ni* (High Five) insect cells (Orbigen, San Diego, CA). Coronavirus reactivity was measured by means of enzyme-linked immunosorbent assays where sera from the study participants were diluted 1:200 and incubated with the nucleocapsid antigens bound to the solid phase using a modified GST-capture method.^{34,38} Negative control antigens included preparations that contained just baculovirus DNA, insect cells, and the GST cloning vector without a nucleocapsid insert.

Statistical Analyses

We expressed results quantitatively as antibody levels and qualitatively as seroprevalence relative to a defined seropositivity cutoff value. To minimize error associated with plate-to-plate variation, the data were mean normalized. Mean normalization was done by adjusting the absorbances for each individual so that mean optical density values of the nonpsychiatric controls on each plate equaled a value of "1." Significant differences between groups in quantitative mean antibody levels were analyzed with 2-tailed t tests. Significant differences in qualitative rates of seropositivity between groups were identified with χ^2 tests (α level = .05). For this qualitative aspect, we generated new seropositivity cutoff values based on the mean-normalized data, and these values differed from those previously generated from raw data in our coronavirus assay development trials.³⁴ New seropositivity cutoff values were defined as follows: 229E, 0.07; HKU1, 0.13; NL63, 0.11; and OC43, 0.19. For continuity with our previous study, we also analyzed our

data based on cutoff methodology using the previously determined cutoff values.³⁴

Significant associations with diagnostic groups were further assessed with multinomial logistic regressions using diagnostic group as the principal outcome variable and quantitative antibody levels as a covariate. Other covariates used for all regressions were age, gender, race, maternal education level, and smoking status. Information regarding maternal education level, which we used to reflect socioeconomic status, was only available for 101 of the 106 individuals with a recent onset of psychotic symptoms.

All analyses were performed with STATA version 10 (STATA Corp LP, College Station, TX).

Results

We found that a recent onset of psychotic symptoms was significantly associated with coronavirus exposure as determined by bivariate analyses of quantitative antibody levels and qualitatively determined seroprevalence. For 3 of the 4 coronaviruses (HKU1, NL63, and OC43), mean antibody levels against each antigen were significantly greater in the recent onset group ($n = 106$) as compared with controls ($n = 196$) (P values ranged from .02 to .00001; t statistics and 2-tailed P values are shown in table 3). In these tests, HKU1 showed significant differences between patients and controls ($P \leq .002$) and NL63 showed highly significant intergroup differences ($P \leq .00001$). Rates of seropositivity in the recent onset population were significantly increased for all human coronaviruses as compared with controls (P values ranged from .009 to .0001; χ^2 statistics and P values are shown in table 4). The greatest difference in seropositivity rates between patients and controls was observed for HKU1 (93.4% vs 77.0%, $P \leq .0001$). Coronavirus seropositivity and antibody levels were increased with both mood and

Table 3. Coronavirus Immunoglobulin G Antibody Levels

	<i>n</i>		229E	HKU1	NL63	OC43
Control	196	Mean ± SEM	1.00 ± 0.05	1.00 ± 0.07	1.00 ± 0.03	1.00 ± 0.05
Recent onset	106	Mean ± SEM	1.05 ± 0.06	1.43 ± 0.14	1.23 ± 0.04	1.18 ± 0.06
		<i>t</i> Statistic	0.62	3.09	4.40	2.33
		<i>P</i> value	.54	.002	.00001	.02
Mood disorders	53	Mean ± SEM	0.95 ± 0.08	1.39 ± 0.19	1.17 ± 0.05	1.16 ± 0.90
		<i>t</i> Statistic	−0.52	2.33	2.42	1.53
		<i>P</i> value	.60	.02	.02	.13
Schizophrenia-spectrum disorders	53	Mean ± SEM	1.15 ± 0.08	1.47 ± 0.20	1.30 ± 0.05	1.21 ± 0.05
		<i>t</i> Statistic	1.48	2.75	4.27	2.10
		<i>P</i> value	.14	.006	.00001	.04

schizophrenia-spectrum disorder diagnoses compared with controls, but coronavirus measures were generally more consistently elevated with a schizophrenia-spectrum disorder diagnosis (*P* values ranged from .00001 to .14) than with a mood disorder diagnosis (*P* values ranged from .01 to .60; tables 3 and 4).

We employed multivariate analyses to examine relationships between diagnosis, antibody levels, and demographic variables. All multivariate models included age, gender, race, maternal education, and smoking status as covariates. Multivariate analyses confirmed the statistically significant association of HKU1 (odds ratio [OR] = 1.32, 95% CI = 1.03–1.67, *P* ≤ .027) and NL63 (OR = 2.42, 95% CI = 1.25–4.66, *P* ≤ .008) antibody levels with a recent onset of psychotic symptoms diagnosis (ORs, *P* values, and CIs are shown in table 5). NL63 antibody levels were further significantly associated with schizophrenia-spectrum disorders compared with controls (OR = 3.10, 95% CI = 1.27–7.58, *P* ≤ .013; table 5). HKU1 antibody levels showed a modest association with mood disorders compared with controls (OR = 1.32, 95% CI = 0.99–1.76, *P* ≤ .053; table 5).

Discussion

In this study, we estimated coronavirus immunoreactivity through measures of antibody levels and seroprevalence and found increased rates of immunoreactivity for certain coronavirus strains in individuals with a recent onset of psychotic symptoms as compared with controls without a history of psychiatric disorder. Of the 4 coronavirus strains tested, the more newly discovered NL63 and HKU1 showed consistent disease-associated significance in all statistical analyses. The conferred risk for neuropsychiatric disease by coronavirus immunoreactivity was modestly elevated as evident by ORs of 1.3 for HKU1 and 2.4 for NL63. When the patient group was broken down into mood and schizophrenia-spectrum disorders, however, the OR for NL63 association with schizophrenia-spectrum disorders increased to 3.1. NL63, in particular, should be the subject of further studies in individuals with schizophrenia to determine if viral infection and symptom onset can be temporally linked.

By assigning patients to schizophrenia-spectrum and mood disorder groups, we may have introduced some confounding elements to the smaller group analyses. Schizoaffective disorder, which has a mood disorder

Table 4. Coronavirus Seropositivity Rates

	<i>n</i>	Seropositivity	229E	HKU1	NL63	OC43
Control	196	<i>n</i> (%)	174 (88.8)	151 (77.0)	184 (93.9)	164 (83.7)
Recent onset	106	<i>n</i> (%)	104 (98.1)	99 (93.4)	106 (100)	100 (94.3)
		χ^2 Statistic	8.20	12.91	6.76	7.12
		<i>P</i> value	.004	.0001	.009	.008
Mood disorders	53	<i>n</i> (%)	51 (96.2)	49 (92.5)	53 (100)	49 (92.5)
		χ^2 Statistic	2.66	6.27	3.41	2.60
		<i>P</i> value	.103	.012	.065	.107
Schizophrenia-spectrum disorders	53	<i>n</i> (%)	53 (100)	50 (94.3)	53 (100)	51 (96.2)
		χ^2 Statistic	6.53	8.02	3.41	5.58
		<i>P</i> value	.011	.005	.065	.018

Table 5. Coronavirus Immunoglobulin G Antibodies and Risk of Recent onset of Psychotic Symptoms

	<i>n</i>		229E	HKU1	NL63	OC43
Recent-onset psychoses	101	OR	1.06	1.32	2.42	1.45
		<i>P</i> value	.780	.027	.008	.078
		CI	0.71–1.59	1.03–1.67	1.25–4.66	0.96–2.20
Mood disorders	51	OR	0.81	1.32	1.92	1.38
		<i>P</i> value	.424	.053	.102	.204
		CI	0.48–1.36	0.99–1.76	0.88–4.22	0.84–2.29
Schizophrenia-spectrum disorders	50	OR	1.43	1.28	3.10	1.55
		<i>P</i> value	.18	.105	.013	.108
		CI	0.85–2.42	0.95–1.75	1.27–7.58	0.91–2.65

Note: Multiple logistic regressions include age, gender, race, maternal education, and smoking status as covariates. OR, odds ratio; CI, confidence interval.

component, accounted for 15% ($n = 8$) of those with schizophrenia-spectrum disorders. Our data show a lack of association of NL63 with mood disorders; therefore, if individuals with schizoaffective disorder were suffering more from the mood component of their disease at the time of the blood draw, then our OR measures are actually conservative estimates for disease association. It is also possible that diagnostic uncertainty present close to the start of an illness with psychotic symptoms may have led to some misclassifications. For example, differentiating bipolar disorder with psychotic features from schizoaffective disorder may not always be 100% accurate. Within-group distributions also may have influenced study outcome in the mood disorder subgrouping where 30.2% ($n = 16$) of the patients suffered from major depression and the remainder from subtypes of bipolar disorder.

Other limitations should also be considered when interpreting the results presented here, including study design and control group representativeness. For each serum sample, the antibodies measured represent an immunological profile based on a single time point, and therefore, data can only be analyzed in a cross sectional manner. Future studies that incorporate a prospective design will allow the assessment of changes in antibody levels over time and will enable us to ascertain the utility of this antibody measure as a diagnostic tool and/or etiological agent. Another potentially limiting factor may be that the inclusion/exclusion criteria for our control group led to a sample that may not be fully representative of persons in the general population. The net effect of our recruitment of volunteers who were excluded from having Axis I disorders, viral infections, or antiviral therapy may in fact be a control group that was unusually healthy. An ideal control group would be composed of individuals with nonpsychotic learning or developmental disorders who live similar lifestyles as those who go on to develop mental disorders with psychotic symptoms. With respect to the exclusion of current viral infections and antiviral medications in the control but not in the patient

group, any virus showing coinfection with the coronaviruses could potentially bias the results in favor of a spurious association. Of note, we performed a similar evaluation of the influenza A and B viruses in these populations, and differences in antibody levels between patients and controls were not detected (data not shown), thus providing some evidence against an intergroup unequal exposure rate hypothesis, at least for respiratory viruses. In the future, additional control groups should be evaluated to determine the prevalence of coronavirus infections in a wide range of human populations. As a starting point, though, use of such a control population here is appropriate because to the authors' knowledge coronaviruses have never before been tested for a serological association with patients with these particular disorders.

Mental illness predisposes an individual to a high rate of medical comorbidity, thus making it difficult to disentangle generally poor health due to a suboptimal living environment or at-risk lifestyle from a disease risk related to exposure to a specific pathogen. It is documented that individuals with mental disorders have increased incidences of cardiovascular disease, diabetes, obesity, hypertriglyceridemia, hepatitis B virus, HIV, and smoking-related illnesses compared with people with no history of neuropsychiatric disorders.^{39–42} We can speculate that environmental effects such as living conditions may less likely be confounding factors in the present study because our cohort is composed of individuals who are relatively young and who have only recently become symptomatic, as compared with people who have been suffering from a serious psychiatric disorder for an extended period. Because subjects in the control group were older, had mothers with fewer years of education, and smoked less, we adjusted our multivariate analyses for factors such as age, socioeconomic status, and smoking. Nevertheless, other epidemiological explanations could account for the increased exposure rates observed for the patient group, with multiple environmental factors likely contributing to comorbid health conditions in those who

are mentally ill. Mental health policy efforts would benefit from data that document the poor physical health of individuals with serious mental disorders.

Data from a diversity of clinical, animal, and cell culture studies support that coronaviruses are neurotropic.^{26–33,43} In people infected with and who have survived SARS, serious neuropsychiatric complications including psychosis have been observed.^{28,32} Auditory and visual hallucinations as well as manic and depression disorders have all been reportedly associated with SARS infections.^{28,32} The extent that neurological problems in SARS patients originate from the virus rather than medications used to treat the infection is not currently understood; however, SARS-specific nucleotide sequences were isolated from cerebrospinal fluid and postmortem brain tissue, suggesting that viral invasion may play a role in ensuing psychiatric complications.^{30,33} Evidence for coronavirus infections of the central nervous system also comes from studies of multiple sclerosis, a disease characterized by nerve demyelination.^{26,29} Reverse transcription-polymerase chain reaction in postmortem brain tissue confirms that in some individuals, OC43 and/or 229E transcripts are present,^{26,29} with one report documenting significant differences in brain coronavirus RNA between cases and controls.²⁶ Studies to evaluate the extent that the more recently discovered coronaviruses, NL63 and HKU1, can invade neuronal cells are warranted.

In summary, results from our study document that coronavirus exposure may be a comorbid risk factor in individuals with serious mental disorders. More investigation is needed to determine if respiratory infection and subsequent neuroinvasion could explain the association of increased coronavirus seroprevalence and the recent onset of psychotic symptoms. It is of note that cinanserin, a serotonin antagonist originally developed for the treatment of schizophrenia, has recently been shown to have the ability to inhibit the replication of a wide range of coronaviruses.^{44,45} A better understanding of the role of coronaviruses in the etiopathogenesis of disorders with psychotic symptoms might lead to new methods for studying, diagnosing, and treating these diseases.

Funding

Stanley Medical Research Institute.

Acknowledgments

The authors thank Barbara Silver, Lin Xue, and Bogdana Krivogorsky for laboratory assistance and Ann Cusic for administrative assistance.

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