ORIGINAL ARTICLE

Increased Prevalence of Diverse *N*-Methyl-D-Aspartate Glutamate Receptor Antibodies in Patients With an Initial Diagnosis of Schizophrenia

Specific Relevance of IgG NR1a Antibodies for Distinction From N-*Methyl-D-Aspartate Glutamate Receptor Encephalitis*

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Context: Evidence for symptomatic convergence of schizophrenia and *N*-methyl-D-aspartate glutamate receptor (NMDA-R) encephalitis highlights the need for an assessment of antibody prevalence and specificity for distinct disease mechanisms in patients with a diagnosis of schizophrenia among glutamatergic pathophysiologic abnormalities in psychiatric disorders.

Objectives: To compare the specificity and prevalence of NMDA-R antibodies in schizophrenia (*DSM-IV* criteria) with those of other psychiatric diagnoses and to determine whether antibody subtypes characterize overlap with and distinction from those in NMDA-R encephalitis.

Design: Serum from 459 patients admitted with acute schizophrenia, major depression (MD), and borderline personality disorder (BLPD) or individuals serving as matched controls was obtained from our scientific blood bank. To explore epitope specificity and antibody subtype, IgA/IgG/IgM NMDA-R (NR1a or NR1a/NR2b) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPA-R) (GluR1/GluR2) serum antibodies were determined.

Participants: Two hundred thirty matched healthy controls were compared with patients (unmedicated for at least 6 weeks) with schizophrenia (n=121), MD (n=70), or BLPD (n=38).

Main Outcome Measures: The primary outcome was the overall number of seropositive cases for NMDA-R and AMPA-R antibodies; the secondary outcome was disease specificity of IgA/IgG/IgM antibodies and epitope specificity for clinical subgroups.

Results: Diverse NMDA-R antibodies were identified in 15 subjects, primarily those with an initial schizophrenia diagnosis (9.9%), opposed to MD (2.8%), BLPD (0), and controls (0.4%). Retrospectively, 2 patients initially classified as having catatonic or disorganized schizophrenia were reclassified as having misdiagnosed NMDA-R encephalitis (presence of specific serum and cerebrospinal fluid IgG NR1a antibodies). In all other seropositive cases, the antibodies consisted of classes IgA and/or IgM or were directed against NR1a/NR2b (not against NR1a alone). None of the patients or controls had antibodies against AMPA-R.

Conclusions: Acutely ill patients with an initial schizophrenia diagnosis show an increased prevalence of NMDA-R antibodies. The repertoire of antibody subtypes in schizophrenia and MD is different from that with NMDA-R encephalitis. The latter disorder should be considered as a differential diagnosis, particularly in young females with acute disorganized behavior or catatonia.

JAMA Psychiatry. 2013;70(3):271-278.
Published online January 23, 2013.
doi:10.1001/2013.jamapsychiatry.86

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Despite decades of research, no unifying pathophysiologic abnormalities have been identified for schizophrenia; consequently, in contrast to other fields of medicine, no physical or laboratory test is available to objectively affirm the diagnosis of schizophrenia. Current diagnostic classification systems (*DSM-IV* and *International Statistical Classification of Diseases, 10th Revision*) rely on psychopathology, social or occupational dysfunction, and time criteria to define a clinical diagnosis of schizophrenia if substance abuse, adverse effects of medication, or psychosis resulting from other medical conditions have been excluded by taking a thorough patient history, as well as performing a physical examination, routine blood tests, brain

imaging, and screening for illegal drugs or alcohol addiction. Thus, as originally developed by Eugen Bleuler,[1] schizophrenia is still a diagnostic construct that describes a clinical syndrome rather than a clearly defined disorder. Notably, Bleuler used the term *schizophrenias*, which referred to the observed clinical heterogeneity that may be paralleled by different underlying pathophysiologic mechanisms.[1] Therefore, the identification of endophenotypes by clinical features or molecular markers may be a promising approach for future psychiatric research to detect clusters of patients with distinct pathophysiologic abnormalities and pave the way for the development of improved treatment strategies, which could progress from symptomatic to causal therapies.

In addition to other established theories, such as the neurodevelopmental, dopamine, or glutamate hypotheses, there is growing evidence for an immune component in a subgroup of patients. For example, several immune-related susceptibility genes for schizophrenia have been identified in the major histocompatibility complex region of chromosome 6p21.3-22.1.[2] Other studies have described changes in the profile of inflammatory cytokines and numeric distribution of lymphocyte subsets in the peripheral blood of patients with schizophrenia.[3,4] A low-grade neuroinflammation hypothesis in schizophrenia is supported by a recent postmortem brain analysis and studies of cerebrospinal fluid (CSF), which identified slightly increased cell counts and moderate blood-brain or blood-CSF barrier dysfunction in patient subgroups.[5-8] Despite such convincing evidence, it remains a challenge to determine how these immunopathologic conditions are associated with disturbances of neurotransmission in schizophrenia.[9]

Antibodies that are directed against disease-relevant brain tissue epitopes in patients with schizophrenia could provide a link between immune abnormalities and altered neurotransmission. Although initial studies[10] were limited by a lack of methodologies to identify specific antigens, more recent publications[11,12] have reported autoantibodies against neurotransmitter receptors, such as the muscarinic acetylcholine receptor. Dalmau and colleagues[13] discovered in patients with encephalitis that schizophrenia-like psychotic and catatonic symptoms could be triggered by IgG serum and CSF antibodies that are directed against extracellular epitopes of the NR1a subunit of *N*-methyl-D-aspartate glutamate receptors (NMDA-R). These antibodies induce a reversible and selective decrease of NMDA-R clusters in postsynaptic dendrites by a mechanism of cross-linking and internalization.[14] Clinically, most patients with NMDA-R encephalitis develop a multistage illness that progresses from psychosis, memory deficits, seizures, and language disintegration to a state of unresponsiveness with catatonic features often associated with abnormal movements, as well as autonomic and breathing instability. The disorder has a potentially relapsing disease course and predominantly affects young females; it is eventually related to the presence of an ovarian teratoma and often responds to tumor resection and immunotherapy (first-line: corticosteroids, intravenous immunoglobulins, plasma exchange; second-line: rituximab or cyclophosphamide).[15,16] Recent publications on NMDA-R encephalitis have focused on patients with the full-blown syndrome; however, mild or incomplete forms of the disorder (formes frustes) with predominant or isolated psychiatric symptoms could occur. It has been suggested that the profile of symptoms in NMDA-R encephalitis depends on the intensity of the antibody effects on the density of NMDA-R,[17] which is similar to observations after ketamine hydrochloride and phencyclidine hydrochloride administration.[18,19] Low doses of these NMDA-R antagonists cause psychosis, anxiety, agitation, memory disturbance, decreased responsiveness to pain, and speech reduction, whereas higher doses produce dissociative anesthesia, a state of profound unresponsiveness with catatonic features, orofacial and limb dyskinesias, autonomic instability, and seizures.[17,20,21]

Interestingly, Zandi et al[22] observed NMDA-R serum antibodies in approximately 6% (3 of 46 patients) with first-onset schizophrenia. The authors emphasized that there were no clinical features differentiating seropositive patients from those with other forms of psychosis and that antibody-positive patients met the clinical *DSM-IV* criteria for schizophrenia. However, the applied test system did not distinguish between different epitopes of the NMDA-R or immunoglobulin classes, and the cohort was not suitable for an investigation of diagnostic subtypes. A larger, clinically diverse cohort is needed to characterize the epitope specificity of antibody subtypes and disambiguate patients with schizophrenia from those with psychotic NMDA-R encephalitis.

Notably, psychotic or psychotiform symptoms, anxiety, agitation, and deficits in cognition can also occur in other diagnostic entities in the field of psychiatry and have been associated with alterations in glutamatergic neurotransmission, such as major depression (MD) or borderline personality disorder (BLPD).[23,24] However, the prevalence of NMDA-R antibodies in these psychiatric disease entities is unknown. There may be similar mechanisms that affect NMDA receptors, and current antidepressant treatment approaches include NMDA-R inhibitors. Therefore, serum samples from unmedicated, acutely ill patients with clinical diagnoses of schizophrenia, MD, and BLPD were needed to characterize the disease specificity of NMDA-R antibodies. To test the clinical application of these potentially relevant biomarkers, a large sample of healthy individuals serving as controls was required to estimate the incidence rates of these putative biomarkers in unaffected individuals.

Acute and severe psychosis in cases of NMDA-R encephalitis is associated with high serum and CSF IgG antibody titers against the NR1a subunit of the NMDA-R.[13] However, psychiatric symptoms may not be exclusively induced by this antibody subtype. Antibodies against different epitopes, such as NR2a and NR2b subunits of the NMDA-R, have been described[25-28] in cases of limbic encephalitis or systemic lupus erythematosus, which affect the central nervous system. For a broad clinical evaluation, the spectrum of immunologic responses and their disease specificities should therefore be explored by the detection of IgG, IgA, and IgM antibodies against NR1a and the heterodimer NR1a/NR2b. Because pure psychiatric manifestations have also been reported in patients with antibodies against α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptors (AMPA-R), we examined the blood

Table 1. Demographic and Clinical Data of Patients and Individuals Who Served as Controls

Characteristic	Mean (SD)			
	Controls (n = 230)	Patients With Schizophrenia (n = 121)	Patients With MD (n = 70)	Patients With BLPD (n = 38)
Age, y	36 (12)	36 (11)	40 (12)	26 (8)
Sex, No.				
Female	71	44	44	24
Male	159	77	26	14
First episode, No.				
Yes	. . .	47	35	10
No	. . .	74	35	28
Illness duration, y[a]	. . .	9 (8)	9 (7)	10 (8)
PANSS P1-P7, score	. . .	22 (6)	9 (2)	8 (2)
PANSS N1-N7, score	. . .	18 (8)	13 (5)	8 (3)
PANSS G1-G16, score	. . .	41 (11)	34 (7)	18 (6)
HAMD-21, score	. . .	17 (9)	21 (7)	14 (9)

Abbreviations: BLPD, borderline personality disorder; HAMD-21, 21-item Hamilton Scale for Depression; MD, major depression; PANSS, Positive and Negative Syndrome Scale.

[a] Refers to patients who were acutely ill and unmedicated but did not belong to the drug-naive first-episode group.

samples for antibodies against the GluR1 and GluR2 subunits of AMPA-R.[29] The primary outcome was the overall number of seropositive cases for NMDA-R and AMPA-R antibodies; the secondary outcome was disease specificity of IgA/IgG/IgM antibodies and epitope specificity for clinical subgroups.

METHODS

SPECIMENS

The study was performed in accordance with German laws, the Declaration of Helsinki, and the guidelines of the local institutional review board. Written informed consent was obtained from all participants. We screened all available serum samples of acutely ill (unmedicated for at least 6 weeks) patients from a recently established scientific blood bank at Magdeburg University's Department of Psychiatry for the presence of different kinds of glutamate receptor antibodies (see the section "Detection of Antibodies Against Glutamate Receptors"). The patients were aged 18 to 60 years and received a clinical diagnosis of schizophrenia (n=121), MD (n=70), or BLPD (n=38) according to *DSM-IV* criteria (**Table 1**).[30] Blood samples from acute disease phases were obtained within 24 hours after admission to Magdeburg University's Department of Psychiatry from June 2007 to May 2010. Individuals serving as matched controls (n=230) were recruited during the same period. Psychiatric abnormalities were monitored by 2 well-trained psychologists using the Positive and Negative Syndrome Scale (PANSS) and the 21-item Hamilton Scale for Depression (HAMD-21). All acutely ill participants were treated as inpatients after admission and underwent therapeutic drug monitoring. Clinicians had access to detailed clinical files, including the medical histories by proxy and referral letters from the general practitioners. Follow-up blood samples were collected after clinical stabilization.

Before definite inclusion of the blood samples into our scientific blood bank, as suggested by the diagnostic guidelines of the German Psychiatric Association,[31] psychosis resulting from other medical conditions and substance-induced psychosis were excluded by a thorough physical examination, routine blood analysis (including differential blood cell count, kidney function tests, and levels of C-reactive protein, glucose, lipids, liver enzymes, and thyroid hormones), screening for illegal drugs, and magnetic resonance imaging of the brain.[30] In addition, electroencephalography (EEG) was performed. The same tests were carried out in the control, MD, and BLPD participants. Patients with a history of immune diseases, immunomodulating treatment, cancer, chronic terminal disease, cardiovascular disorders, diabetes mellitus, substance abuse, and severe trauma or clinical/paraclinical findings indicating these disorders were excluded, and controls were screened for personal or family history of neuropsychiatric disorders using the Mini-International Neuropsychiatric Interview.[32]

Blood was collected between 8 and 9 AM (fasted, 2-hour clotted). Diagnostic CSF specimens were obtained by routine lumbar puncture in all patients with serum IgG NMDA-R autoantibodies. Serum and CSF samples were centrifuged at 2000*g* for 10 minutes and stored at –80°C until analysis. Identical storage procedures were used for patient and control samples.

DETECTION OF ANTIBODIES AGAINST GLUTAMATE RECEPTORS

Antibody testing of all samples was performed at the Institute for Experimental Immunology in Lübeck, using a standardized laboratory assay as previously described.[33,34] Patients and controls were randomly interspersed during the course of testing. Briefly, plasmids that contained glutamate receptor-type NMDA (subunits NR1a/NR1a and NR1a/NR2b)[15] or type AMPA (subunits GluR1 and GluR2)[29] were transfected into HEK293 cells. Recombinant cells were grown on cover glasses and fixed with acetone. Coated cover glasses were cut into millimeter-sized fragments (biochips) and used side-by-side with fragments containing untransfected cells as substrates in the indirect immunofluorescence test. The slides were incubated with patient samples at a starting dilution of 1:10 (serum) or undiluted (CSF). After incubation for 30 minutes at room temperature, the slides were washed in phosphate-buffered saline (PBS)–Tween for 5 minutes. In separate analyses, bound antibodies were labeled using fluorescein-conjugated goat antihuman IgG, IgA, or IgM antibodies for 30 minutes and washed in PBS-Tween for 5 minutes. Phosphate-buffered saline buffered glycerol (containing triethylenediamine to reduce bleaching) was added and a coverglass was applied. Samples were classified as positive or negative on the basis of the intensity of the specific immunofluorescence of transfected cells in direct comparison

Table 2. Demographic Data and Diagnostic Test Results of NMDA-R–Seropositive Cases

Case No./ Sex/Age, y	Diagnosis[a]	Illness Duration	MRI	EEG	Serum Antibody Titer			Epitope
					IgA	IgG	IgM	
1/F/25	Disorganized SCZ → NMDA-R encephalitis	First episode	Normal	Irregular alpha-EEG	100	1000	Negative	NR1a
2/F/18	Catatonic SCZ → NMDA-R encephalitis	First episode	Normal	Irregular alpha-EEG, intermittent diffuse theta activity	Negative	3200	Negative	NR1a
3/M/24	Paranoid SCZ	First episode	Normal	Irregular alpha-EEG	Negative	Negative	10	NR1a
4/F/35	Paranoid SCZ	First episode	Normal	Alpha-EEG, 12/s	Negative	Negative	100	NR1a
5/M/22	Paranoid SCZ	First episode	Normal	Beta-EEG	100	Negative	Negative	NR1a
6/F/44	Paranoid SCZ	3 y	Minor bifrontal atrophy	Irregular alpha-EEG, intermittent diffuse theta activity	320	Negative	Negative	NR1a
7/F/60	Paranoid SCZ	10 y	Normal	Alpha-EEG, 10/s	320	Negative	Negative	NR1a
8/M/45	Paranoid SCZ	13 y	Normal	Alpha-EEG, 10/s	320	Negative	32	NR1a
9/M/38	Paranoid SCZ	14 y	Minor bifrontal atrophy	Alpha-EEG, 10/s	Negative	Negative	100	NR1a
10/M/44	Paranoid SCZ	19 y	Few unspecific T2 white matter lesions	Alpha-EEG, 8/s	Negative	320	Negative	NR1a/NR2b[b]
11/M/58	Paranoid SCZ	20 y	Normal	Beta-EEG	100	Negative	Negative	NR1a
12/M/50	Paranoid SCZ	21 y	Normal	Alpha-EEG, 10/s	Negative	100	Negative	NR1a/NR2b[b]
13/M/54	MD	First episode	Minor cortical and subcortical atrophy	Alpha-EEG, 8/s	32	Negative	Negative	NR1a/NR2b[b]
14/M/44	MD	First episode	Normal	Alpha-EEG, 9/s	320	Negative	Negative	NR1a
15/M/22	Control	. . .	Normal	Alpha-EEG, 10/s	Negative	Negative	100	NR1a

Abbreviations: EEG, electroencephalography; MD, major depression; MRI, magnetic resonance imaging; NMDA-R, *N*-methyl-D-aspartate glutamate receptor; SCZ, schizophrenia.

[a] In patients 1 and 2, → indicates an initial schizophrenia diagnosis that was reclassified post hoc in our study as NMDA-R encephalitis resulting from the detection of specific serum and cerebrospinal fluid IgG NR1a antibodies.

[b] Antibodies against NR1a/NR2b were not reactive against NR1a alone.

with nontransfected cells and control samples. The titer of an antibody was defined as the maximum dilution at which immunoreactivity was visible. All test results were confirmed in a blinded separate analysis.

STATISTICAL ANALYSIS

The diagnostic group differences regarding the distribution of cases with positive antibody titers were calculated by the Yates χ^2 test. Patients 1 and 2 were initially classified as having disorganized or catatonic schizophrenia. However, our study of blood samples from their first clinical evaluation showed post hoc typical features of NMDA-R encephalitis, such as specific IgG NR1a antibodies in the serum and CSF (post hoc diagnostic reclassification) (**Table 2** and eAppendix; http://www.jamapsych.com). We performed an additional data analysis excluding these patients to underpin our findings regarding the diagnosis-specific prevalence of different NMDA-R antibodies. Statistical significance was defined as $P < .05$.

RESULTS

ANALYSIS OF SERUM ANTIBODIES AGAINST NMDA AND AMPA RECEPTORS IN THE WHOLE SAMPLE

Several types (IgA, IgG, and IgM class, directed against NR1a alone or against NR1a/2b) of NMDA-R serum antibodies, were present in 9.9% of the acutely ill patients (12 of 121) with an initial diagnosis of schizophrenia. By contrast, none of the BLPD patients, including 5 with psychotiform symptoms, demonstrated seropositivity. Two of 70 patients (2.8%) in the MD group without psychotic symptoms and 1 of 230 control participants (0.4%) were seropositive (Table 2). The diagnostic group difference was statistically significant (Yates $\chi^2_{3,459} = 20.34$, $P < .001$; even without patients 1 and 2, who were reclassified post hoc as having NMDA-R encephalitis: Yates $\chi^2_{3,457} = 15.82$, $P = .001$), because of a higher relative frequency of cases with NMDA-R antibodies in the schizophrenia cohort (Yates $\chi^2_{1,459} = 20.22$, $P < .001$; without patients 1 and 2: Yates $\chi^2_{1,457} = 16.13$, $P < .001$). No seasonal effects were observed regarding the presence of NMDA-R antibodies during the collection period from 2007 to 2011. The AMPA-1 and AMPA-2 receptor antibodies were negative in all of the tested participants.

IMMUNOGLOBULIN AND EPITOPE SPECIFICITY IN SEROPOSITIVE CASES

The IgG class antibodies directed against NR1a were found in only 2 patients (cases 1 and 2) with an initial diagnosis of disorganized or catatonic schizophrenia. According to Dalmau et al,[17] they could be reclassified post hoc as having NMDA-R encephalitis (eAppendix). Both individuals showed high initial IgG titers (≥1:1000) (eAppendix). In contrast, 2 other individuals with IgG antibodies (cases 10 and 12) received diagnoses of paranoid schizophrenia. They had lower serum antibody titers (≤1:

320), and the antibodies did not bind to NR1a but were reactive only with NR1a/NR2b. In all other seropositive cases, antibodies directed against NR1a alone belonged to the IgA and/or IgM subtype: 5 schizophrenia cases had IgA antibodies and 3 had IgM antibodies. None of the 3 other individuals (cases 13-15) had IgG antibodies against NR1a. Two of these cases had IgA antibodies (MD) and 1 individual (control) had IgM antibodies.

PARACLINICAL CHARACTERIZATION OF ANTI–NMDA-R–SEROPOSITIVE CASES

CSF and Follow-up in Patients With IgG Seropositivity

Positive CSF titers were found in only the 2 patients with IgG NR1a seropositivity (cases 1 and 2; reclassified as NMDA-R encephalitis). During remission, antibody titers declined from 1:320 to 1:32 in the CSF of both patients, which was paralleled by diminished serum IgG NR1a antibody titers (eAppendix). In patient 1, initial lymphocytic pleocytosis (21 cells/μL) and an IgG index of 0.8 normalized during clinical stabilization; however, oligoclonal bands were still present at the final follow-up. Patient 2 had a CSF cell count of 3 cells/μL, an IgG index of 0.8, and positive oligoclonal bands during acute illness. Clinical stabilization was associated with a reduction of inflammatory parameters (1 cell/μL; IgG index, 0.5; and no oligoclonal bands). In contrast, patients 10 and 12 had NR1a/NR2b antibodies in the serum but not in the CSF; both patients had a normal CSF cell count and IgG index; however, patient 10 was positive for oligoclonal bands. As in cases 1 and 2, serum antibody titers decreased along with clinical improvement (eAppendix).

EEG and Magnetic Resonance Imaging

Results of the EEGs performed during the acute stage of the illness were abnormal in cases 1, 2, and 6, showing nonspecific slow and disorganized activity without epileptiform discharges (Table 2). Despite the drastic clinical symptoms and the persistence of antibodies for several years, the brain magnetic resonance imaging results were unremarkable in cases 1 and 2 and in 80.0% (12 of 15 patients) of all anti–NMDA-R–seropositive cases (Table 2). The remaining 3 cases revealed nonspecific changes, including mild bifrontal or general cortical and subcortical atrophy (patients 6, 9, and 13) or nonspecific T2 white matter lesions (patient 10).

COMMENT

To our knowledge, this study of 459 individuals is the largest systematic analysis of glutamate receptor antibodies that focused on acutely ill unmedicated psychiatric patients with different *DSM-IV* diagnoses. Furthermore, this study discerned antibody classes (IgA, IgG, and IgM) and epitopes (NR1a alone vs NR1a/NR2b). Although AMPA-1 and AMPA-2 receptor antibodies were absent in all of the tested participants, we identified several types of serum NMDA-R antibodies in up to 10% of all patients with an initial diagnosis of schizophrenia, but only in approximately 3% of the MD cohort and in none of the BLPD group. Thus, we provide the first evidence of a specifically increased prevalence of NMDA-R antibodies in patients with a schizophrenia diagnosis.

The relevance of the NMDA-R autoimmune responses seems to follow several hierarchical levels of importance. First, despite the increased prevalence of NMDA-R antibodies in patients with an initial diagnosis of schizophrenia, seropositivity was also observed in 2 MD patients and in 1 healthy control participant. Among seropositive individuals, patients 1 and 2 had specific IgG antibodies directed against NR1a, which were also increased in the CSF. These patients had initial diagnoses of disorganized or catatonic schizophrenia; however, retrospectively, it was clear that they had misdiagnosed NMDA-R encephalitis (according to the criteria established by Dalmau et al[17]). Accordingly, paraclinical test results showed slow and disorganized EEG activity during the second disease episode of patient 1, as well as mild lymphocytic pleocytosis and an increased IgG index in the CSF of both patients (eAppendix). In contrast, the IgG NMDA-R antibodies in patients 10 and 12 were probably directed against a different epitope of the NMDA-R, since they were reactive against NR1a/NR2b but not against NR1a alone. Moreover, these patients did not exhibit the characteristic clinical symptoms and inflammatory CSF findings of NMDA-R encephalitis. We conclude from these findings that the clinical manifestation may vary depending on the kind of subunit blockade caused by these autoantibodies. However, epitope mapping experiments and larger numbers of cases are warranted to address this hypothesis. An analysis of NR2a antibodies could have resulted in even more comprehensive data. However, the analysis of this antibody subcategory was not part of the study protocol.

IgA and IgM anti–NMDA-R antibodies were present in all other seropositive cases. These were not specific for schizophrenia, and their significance remains to be clarified, for example, by including CSF samples from patients with acute episodes of neuropsychiatric disease. The eTable shows that there was no clear association pattern regarding the presence of these immunoglobulin classes with disease duration or disease stage. We did not set up B-cell class switch as a hypothesis, since it is known from several autoimmune disorders that IgA or IgM antibodies are not restricted to distinct phases of the disease course but can persistently be found.[35-37] The IgA antibody is typically considered to be of importance on mucosal surfaces. However, persistence in serum as well as intrathecal synthesis of IgA NMDA-R antibodies has been described as markers of synaptic immunity in patients with slow progressive cognitive impairment (clinical symptoms improved after immunotherapy).[38] Thus, the increased prevalence of IgA NMDA-R antibodies in the schizophrenia cohort suggests an immunological link to Emil Kraepelin's "dementia praecox" concept.[39] This idea may be clarified in future studies by providing additional cognitive test data from patients seropositive for IgA NMDA-R antibodies. Notably, NMDA glutamate receptors are also present outside the brain (eg, heart, kid-

ney, and lungs).[40,41] Therefore, systemic immune alterations must be considered when studying the complexity of NMDA-R seropositivity in schizophrenia.[9,42] The contribution of peripheral tissues to the development of autoantibody production and the largely unknown relationship between peripheral tissues and abnormal brain function warrant further investigation. Our study additionally revealed the important insight that NMDA-R antibodies may be detected in healthy individuals (IgM subtype, case 15). It is unknown whether such individuals are prone to a higher risk of developing neuropsychiatric disorders.

LIMITATIONS AND PERSPECTIVES

According to Dalmau et al,[15,17] a specific immune profile can be assumed for NMDA-R encephalitis (IgG NR1a antibodies in serum and CSF), whereas patients with schizophrenia show a less-specific NMDA-R immune response by an ample repertoire of immunoglobulins that are also present in other disorders (cognitive decline, MD). Therefore, the identification of 2 cases of NMDA-R encephalitis in our schizophrenia cohort may appear intriguing. However, we chose a different approach: the primary objective of our study was to compare the prevalence of different kinds of NMDA-R antibodies by an unbiased screening of all available serum samples from our scientific blood bank across 3 patient populations (acutely ill unmedicated schizophrenia, MD, and BLPD) with diagnoses determined using state-of-the-art procedures within the clinical setting of a psychiatric university department. We detected specific IgG NR1a antibodies post hoc and not during the routine diagnostic procedures. Nevertheless, we observed similar levels of significance regarding the overall prevalence of different kinds of NMDA-R antibodies in the schizophrenia cohort, even when we removed cases 1 and 2 from statistical analysis (see the "Results" section). The presentation of this additional statistical analysis does not reflect that we would necessarily prefer one opinion (our unbiased antibody screening approach with post hoc reclassification of 2 cases with an initial schizophrenia diagnosis vs the approach to consider NMDA-R encephalitis from the initial examination as a distinct diagnostic entity). The 2 estimates of significantly increased prevalence in schizophrenia are neither contradictory nor different in their message. We acknowledge that the diagnostic accuracy in psychiatry needs to be understood within the dynamics of ongoing discussions regarding the development of future diagnostic classification systems (*DSM-5* and *International Statistical Classification of Diseases, 11th Revision*) and evolving biomarker research. The latter field is gradually being accepted and aims to identify more objective parameters in the diagnosis of schizophrenia (eg, compared with patient interviews) to increase the reliability of diagnoses among specialists.[43,44] Moreover, the detection of molecular biomarker clusters may be helpful for the identification of distinct underlying abnormalities within the group of schizophrenic psychoses, which would facilitate the development of personalized medicine.

Unfortunately, reliable information on the exact onset of symptoms during the current disease episode was not available for all patients, since initial symptoms appeared before hospitalization and were very dependent from subjective reports. However, given the course of immunologic mechanisms, this information might be highly relevant for future studies.

The putative pathogenic role of these NMDA-R antibodies notwithstanding, their sole presence does not prove their etiologic involvement in schizophrenia. Nevertheless, a pathologic process that resulted in neuronal damage may have caused the immunologic sensitization to NMDA-R as a by-product. However, the associated clinical peculiarities support at least the specificity for this diagnostic group.

CONCLUSIONS

We revealed an increased prevalence of several types of NMDA-R antibodies, affecting up to 10% of patients with an initial clinical diagnosis of schizophrenia. Although this observation corresponds with downstream mechanisms of the glutamate hypothesis in schizophrenia, a novel and clinically distinct group of psychotic patients with specific treatment options should be considered. We confirm evidence for the generally accepted NMDA-R encephalitis-related psychosis according to Dalmau et al[15] and assume that some individuals, particularly young female patients, with disorganized and catatonic features of schizophrenia may be misdiagnosed encephalitis cases.[45,46] However, larger cohorts need to be analyzed, including more patients with catatonic symptoms, to substantiate this conclusion with sufficient statistical power.

Our findings suggest that the repertoire of antibody classes (IgA, IgG, and IgM) and epitope targets (NR1a alone vs NR1a/NR2b) is wider in patients with the psychiatric diagnoses of schizophrenia, MD, or BLPD than in patients with NMDA-R encephalitis (specific IgG NR1a antibodies in serum and CSF). It is unclear whether the increased prevalence of other anti–NMDA-R antibody subtypes in schizophrenia is an epiphenomenon or a weak etiologic feature that is related to the glutamate hypothesis.

In the context of immunologic models of schizophrenia, our findings may provide translational validation to NMDA antagonist-induced alterations that have been observed in preclinical models.[18] The identification of a subcohort with NMDA-R antibodies may open the door to personalized medicine and render patients susceptible to new specific glutamate-modulating, anti-inflammatory, or immunomodulating therapies.

Submitted for Publication: February 10, 2012; final revision received June 8, 2012; accepted July 23, 2012.
Published Online: January 23, 2013. doi:10.1001/2013.jamapsychiatry.86
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Author Contributions: Drs Steiner and Walter and Prof Stoecker contributed equally to the study.

Conflict of Interest Disclosures: Ms Klingbeil, Dr Wandinger, and Prof Stoecker are full-time employees of and hold stock in Euroimmun AG.

Online-Only Material: The eAppendix and eTable are available at http://www.jamapsych.com.

Additional Contributions: Pembroke College (University of Cambridge, Cambridge, UK) has invited Dr Steiner for a Visiting Scholarship. Dominik Albrecht, MD, Henrik Dobrowolny, Anke Dudeck, Daniela Fenker, PhD, Sieglinde Funke, Bernd Hahndorf, MD, Bianca Jerzykiewicz, Gisela Kondratjuk, MD, Gabriela Meyer-Lotz, Katrin Paelchen, Jeanette Schadow, PhD, Renate Stauch, Sabine Westphal, MD, and Kerstin Ziegner, MD, participated in the sample characterization and collection. Josep Dalmau, MD (Department of Neurology, University of Pennsylvania, Philadelphia, PA, USA), provided helpful discussions and advice regarding the manuscript preparation.

REFERENCES

1. Bleuler E. Dementia praecox oder die Gruppe der Schizophrenien. In: Aschaffenburg G, ed. *Handbuch der Psychiatrie*. Leipzig, Germany: Deuticke; 1911.
2. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, Werge T, Pietiläinen OP, Mors O, Mortensen PB, Sigurdsson E, Gustafsson O, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Suvisaari J, Lonnqvist J, Paunio T, Børglum AD, Hartmann A, Fink-Jensen A, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Böttcher Y, Olesen J, Breuer R, Möller HJ, Giegling I, Rasmussen HB, Timm S, Mattheisen M, Bitter I, Réthelyi JM, Magnusdottir BB, Sigmundsson T, Olason P, Masson G, Gulcher JR, Haraldsson M, Fossdal R, Thorgeirsson TE, Thorsteinsdottir U, Ruggeri M, Tosato S, Franke B, Strengman E, Kiemeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Sanjuan J, de Frutos R, Bramon E, Vassos E, Fraser G, Ettinger U, Picchioni M, Walker N, Toulopoulou T, Need AC, Ge D, Yoon JL, Shianna KV, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Carracedo A, Arango C, Costas J, Jönsson EG, Terenius L, Agartz I, Petursson H, Nöthen MM, Rietschel M, Matthews PM, Muglia P, Peltonen L, St Clair D, Goldstein DB, Stefansson K, Collier DA; Genetic Risk and Outcome in Psychosis (GROUP). Common variants conferring risk of schizophrenia. *Nature*. 2009;460(7256):744-747.
3. Steiner J, Jacobs R, Panteli B, Brauner M, Schiltz K, Bahn S, Herberth M, Westphal S, Gos T, Walter M, Bernstein HG, Myint AM, Bogerts B. Acute schizophrenia is accompanied by reduced T cell and increased B cell immunity. *Eur Arch Psychiatry Clin Neurosci*. 2010;260(7):509-518.
4. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. *Biol Psychiatry*. 2008;63(8):801-808.
5. Nikkilä HV, Müller K, Ahokas A, Rimón R, Andersson LC. Increased frequency of activated lymphocytes in the cerebrospinal fluid of patients with acute schizophrenia. *Schizophr Res*. 2001;49(1-2):99-105.
6. Maxeiner HG, Rojewski MT, Schmitt A, Tumani H, Bechter K, Schmitt M. Flow cytometric analysis of T cell subsets in paired samples of cerebrospinal fluid and peripheral blood from patients with neurological and psychiatric disorders. *Brain Behav Immun*. 2009;23(1):134-142.
7. Bechter K, Reiber H, Herzog S, Fuchs D, Tumani H, Maxeiner HG. Cerebrospinal fluid analysis in affective and schizophrenic spectrum disorders: identification of subgroups with immune responses and blood-CSF barrier dysfunction. *J Psychiatr Res*. 2010;44(5):321-330.
8. Busse S, Busse M, Schiltz K, Bielau H, Gos T, Brisch R, Mawrin C, Schmitt A, Jordan W, Muller UJ, Bernstein HG, Bogerts B, Steiner J. Different distribution patterns of lymphocytes and microglia in the hippocampus of patients with residual versus paranoid schizophrenia: further evidence for disease course-related immune alterations? *Brain Behav Immun*. 2012;26(8):1273-1279.
9. Steiner J, Bogerts B, Sarnyai Z, Walter M, Gos T, Bernstein HG, Myint AM. Bridging the gap between the immune and glutamate hypotheses of schizophrenia and major depression: potential role of glial NMDA receptor modulators and impaired blood-brain barrier integrity. *World J Biol Psychiatry*. 2012;13(7):482-492.
10. Henneberg AE, Horter S, Ruffert S. Increased prevalence of antibrain antibodies in the sera from schizophrenic patients. *Schizophr Res*. 1994;14(1):15-22.
11. Borda T, Perez Rivera R, Joensen L, Gomez RM, Sterin-Borda L. Antibodies against cerebral M1 cholinergic muscarinic receptor from schizophrenic patients: molecular interaction. *J Immunol*. 2002;168(7):3667-3674.
12. Tanaka S, Matsunaga H, Kimura M, Tatsumi K, Hidaka Y, Takano T, Uema T, Takeda M, Amino N. Autoantibodies against four kinds of neurotransmitter receptors in psychiatric disorders. *J Neuroimmunol*. 2003;141(1-2):155-164.
13. Dalmau J, Tüzün E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR. Paraneoplastic anti-*N*-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol*. 2007;61(1):25-36.
14. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, Parsons TD, Lynch DR, Dalmau J, Balice-Gordon RJ. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci*. 2010;30(17):5866-5875.
15. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, Dessain SK, Rosenfeld MR, Balice-Gordon R, Lynch DR. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol*. 2008;7(12):1091-1098.
16. Chapman MR, Vause HE. Anti–NMDA receptor encephalitis: diagnosis, psychiatric presentation, and treatment. *Am J Psychiatry*. 2011;168(3):245-251.
17. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol*. 2011;10(1):63-74.
18. Gunduz-Bruce H. The acute effects of NMDA antagonism: from the rodent to the human brain. *Brain Res Rev*. 2009;60(2):279-286.
19. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry*. 1994;51(3):199-214.
20. Weiner AL, Vieira L, McKay CA, Bayer MJ. Ketamine abusers presenting to the emergency department: a case series. *J Emerg Med*. 2000;18(4):447-451.
21. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991;148(10):1301-1308.
22. Zandi MS, Irani SR, Lang B, Waters P, Jones PB, McKenna P, Coles AJ, Vincent A, Lennox BR. Disease-relevant autoantibodies in first episode schizophrenia. *J Neurol*. 2011;258(4):686-688.
23. Walter M, Henning A, Grimm S, Beck J, Schulte RF, Dydak U, Schnepf B, Boeker H, Boesinger P, Northoff G. The relationship between aberrant neuronal activation patterns in the pregenual anterior cingulate, altered glutamatergic metabolism and anhedonia in major depression. *Arch Gen Psychiatry*. 2009;66(5):478-486.
24. Grosjean B, Tsai GE. NMDA neurotransmission as a critical mediator of borderline personality disorder. *J Psychiatry Neurosci*. 2007;32(2):103-115.
25. Arinuma Y, Yanagida T, Hirohata S. Association of cerebrospinal fluid anti-NR2 glutamate receptor antibodies with diffuse neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum*. 2008;58(4):1130-1135.
26. Gono T, Kawaguchi Y, Kaneko H, Nishimura K, Hanaoka M, Kataoka S, Okamoto Y, Katsumata Y, Yamanaka H. Anti-NR2A antibody as a predictor for neuropsychiatric systemic lupus erythematosus. *Rheumatology (Oxford)*. 2011;50(9):1578-1585.
27. Mochizuki Y, Mizutani T, Isozaki E, Ohtake T, Takahashi Y. Acute limbic encephalitis: a new entity? *Neurosci Lett*. 2006;394(1):5-8.
28. Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol*. 2011;10(8):759-772.
29. Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, Matà S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. *Ann Neurol*. 2009;65(4):424-434.
30. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed, text revision. Washington, DC: American Psychiatric Association; 2000.

31. Gaebel W, Falkai P. *Interdisciplinary Praxis Guidelines: AWMF S3 Guidelines of the German Psychiatric Association for Schizophrenia*. Darmstadt, Germany: Steinkopff; 2005.
32. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for *DSM-IV* and *ICD-10*. *J Clin Psychiatry*. 1998;59(suppl 20):22-33.
33. Wandinger KP, Saschenbrecker S, Stoecker W, Dalmau J. Anti–NMDA-receptor encephalitis: a severe, multistage, treatable disorder presenting with psychosis. *J Neuroimmunol*. 2011;231(1-2):86-91.
34. Stöcker K, Stöcker W, Ritter-Frank Y, Scriba PC. Chemically activated glass slides for frozen sections and their use in autoantibody diagnosis. *Acta Histochem Suppl*. 1985;31:283-294.
35. Green PH, Cellier C. Celiac disease. *N Engl J Med*. 2007;357(17):1731-1743.
36. Kasperkiewicz M, Meier M, Zillikens D, Schmidt E. Linear IgA disease: successful application of immunoadsorption and review of the literature. *Dermatology*. 2010;220(3):259-263.
37. Hashimoto T. Immunopathology of IgA pemphigus. *Clin Dermatol*. 2001;19(6):683-689.
38. Prüss H, Höltje M, Maier N, Gomez A, Buchert R, Harms L, Ahnert-Hilger G, Schmitz D, Terborg C, Kopp U, Klingbeil C, Probst C, Kohler S, Schwab JM, Stoecker W, Dalmau J, Wandinger KP. IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment. *Neurology*. 2012;78(22):1743-1753.
39. Kraepelin E. *Dementia Praecox and Paraphrenia*. 8th ed. Edinburgh, Scotland: J & J Gray & Co; 1919.
40. Said SI, Berisha HI, Pakbaz H. Excitotoxicity in the lung: *N*-methyl-D-aspartate–induced, nitric oxide–dependent, pulmonary edema is attenuated by vasoactive intestinal peptide and by inhibitors of poly(ADP-ribose) polymerase. *Proc Natl Acad Sci U S A*. 1996;93(10):4688-4692.
41. Leung JC, Travis BR, Verlander JW, Sandhu SK, Yang SG, Zea AH, Weiner ID, Silverstein DM. Expression and developmental regulation of the NMDA receptor subunits in the kidney and cardiovascular system. *Am J Physiol Regul Integr Comp Physiol*. 2002;283(4):R964-R971.
42. Kirkpatrick B. Schizophrenia as a systemic disease. *Schizophr Bull*. 2009;35(2):381-382.
43. Schwarz E, Guest PC, Rahmoune H, Harris LW, Wang L, Leweke FM, Rothermundt M, Bogerts B, Koethe D, Kranaster L, Ohrmann P, Suslow T, McAllister G, Spain M, Barnes A, van Beveren NJ, Baron-Cohen S, Steiner J, Torrey FE, Yolken RH, Bahn S. Identification of a biological signature for schizophrenia in serum. *Mol Psychiatry*. 2012;17(5):494-502.
44. Schwarz E, Izmailov R, Spain M, Barnes A, Mapes JP, Guest PC, Rahmoune H, Pietsch S, Leweke FM, Rothermundt M, Steiner J, Koethe D, Kranaster L, Ohrmann P, Suslow T, Levin Y, Bogerts B, van Beveren NJ, McAllister G, Weber N, Niebuhr D, Cowan D, Yolken RH, Bahn S. Validation of a blood-based laboratory test to aid in the confirmation of a diagnosis of schizophrenia. *Biomark Insights*. 2010;5:39-47.
45. Fink M, Taylor MA. The catatonia syndrome: forgotten but not gone. *Arch Gen Psychiatry*. 2009;66(11):1173-1177.
46. Tsutsui K, Kanbayashi T, Tanaka K, Boku S, Ito W, Tokunaga J, Mori A, Hishikawa Y, Shimizu T, Nishino S. Anti–NMDA-receptor antibody detected in encephalitis, schizophrenia, and narcolepsy with psychotic features. *BMC Psychiatry*. May 8, 2012;12:37. doi:10.1186/1471-244X-12-37.

Announcement

With regret, I announce the retirements of William (Will) T. Carpenter, MD, and Fritz A. Henn, MD, PhD, from the Editorial Board of *JAMA Psychiatry*. In aggregate, they have served more than 50 years on the board, which speaks to the wisdom and generosity of spirit they provided to the publication's editors.

I am pleased to announce the appointment of 2 new members. To bolster our statistical expertise, Robert D. Gibbons, PhD, the director of the Center for Health Statistics at the University of Chicago and professor of medicine, health studies, and psychiatry, will join the board. To strengthen our bench in brain imaging and developmental neuroscience, Stephen R. Dager, MD, professor of radiology and bioengineering at the University of Washington School of Medicine, will join the board. I look forward to working with Robert and Steve, as well as the rest of the Editorial Board, as we integrate *JAMA Psychiatry* into The JAMA Network.

Joseph T. Coyle, MD, Editor