

🥑 💽 Prevalence and clinical characteristics of serum neuronal cell surface antibodies in first-episode psychosis: a case-control study

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Summary

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Background Psychosis is a common presenting feature in antibody-mediated encephalitis, for which prompt recognition and treatment usually leads to remission. We aimed to investigate whether people with circumscribed schizophrenia-like illnesses have such antibodies-especially antibodies against the N-methyl-D-aspartate receptor (NMDAR)-more commonly than do healthy controls.

Methods We recruited patients aged 14-35 years presenting to any of 35 mental health services sites across England with first-episode psychosis, less than 6 weeks of treatment with antipsychotic medication, and a score of 4 or more on at least one selected Positive and Negative Syndrome Scale (PANSS) item. Patients and controls provided venous blood samples. We completed standardised symptom rating scales (PANSS, ACE-III, GAF) at baseline, and tested serum samples for antibodies against NMDAR, LGI1, CASPR2, the GABA, receptor, and the AMPA receptor using live cell-based assays. Treating clinicians assessed outcomes of ICD diagnosis and functioning (GAF) at 6 months. We included healthy controls from the general population, recruited as part of another study in Cambridge, UK.

Findings Between Feb 1, 2013, and Aug 31, 2014, we enrolled 228 patients with first-episode psychosis and 105 healthy controls. 20 (9%) of 228 patients had serum antibodies against one or more of the neuronal cell surface antibodies compared with four (4%) of 105 controls (unadjusted odds ratio 2.4, 95% CI 0.8-7.3). These associations remained non-significant when adjusted for current cigarette smoking, alcohol consumption, and illicit drug use. Seven (3%) patients had NMDAR antibodies compared with no controls (p=0.0204). The other antibodies did not differ between groups. Antibody-positive patients had lower PANSS positive, PANSS total, and catatonia scores than did antibodynegative patients. Patients had comparable scores on other PANSS items, ACE-III, and GAF at baseline, with no difference in outcomes at 6 months.

Interpretation Some patients with first-episode psychosis had antibodies against NMDAR that might be relevant to their illness, but did not differ from patients without NMDAR antibodies in clinical characteristics. Our study suggests that the only way to detect patients with these potentially pathogenic antibodies is to screen all patients with first-episode psychosis at first presentation.

Funding Medical Research Council.

Introduction

Autoantibodies to neuronal cell surface receptors and related proteins have been described in association with encephalitic syndromes that frequently include psychiatric symptoms-usually psychosis-as a prominent part of the phenotype. Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, first described in 2007,1 is the most common of these. It is a multistage encephalitis caused by antibodies to the GluN1 (NR1) subunit of the NMDAR. NMDAR encephalitis presents initially with psychiatric symptoms in more than two thirds of patients before recognition of neurological symptoms including cognitive deficits, seizures, autonomic instability, and movement disorder.²

Several autoimmune encephalitides have been identified associated with autoantibodies to other cell surface antigens, including leucine-rich glioma inactivated 1 (LGI1),3 contactin associated protein 2 (CASPR2),³ the α-amino-3-hydroxy-5methyl-4-isoxazole-propionic acid receptor (AMPAR),4 and the y-aminobutyric-acid receptor (GABA, R).5 Psychiatric or behavioural manifestations are commonly described, and occasional cases of patients with purely psychiatric presentations with these antibodies have been reported.5,6

In autoimmune encephalitis associated with voltagegated potassium channel (VGKC)-complex antibodies, the antibodies are usually directed against the neuronal surface antigens LGI1 and CASPR2, which are components of the VGKC-complex; these antibodies are thought to be pathogenic.3 In other cases, the VGKCcomplex antibodies do not bind to LGI1 or CASPR2,78 and might bind to intracellular, non-pathogenic epitopes on the VGKC-complex; these antibodies, therefore, are unlikely to be causative. However, one study8 in children suggested that these antibodies might be a marker for immune-responsive neuroinflammatory conditions.

Research in context

Evidence before this study

We searched MEDLINE for studies published from Jan 1, 2001, to Aug 31, 2016, on the prevalence of neuronal cell surface antibodies in patients with psychosis. We used search terms "antibod*" AND "psychosis" OR "schiz*" AND "NMDA*" OR "N-methyl-D-aspartate receptor" OR "LGI1" OR "CASPR2" OR "mGluR5" OR "AMPA*" OR "GABAA*" OR "GABAB". We excluded non-English language articles. Several studies have examined the prevalence of serum NMDAR antibodies, with one systematic review and meta-analysis of nine studies-including 3387 participants—showing three times greater (odds ratio 3.1) NMDAR antibody positivity in patients with schizophrenia or schizoaffective disorder, bipolar affective disorder, or major depressive disorder compared with controls. Subsequent studies have reported varying results. One study in children found five (12%) of 43 children with psychosis with NMDAR IgG antibodies, and no such antibodies in 43 healthy or illness controls. The largest studies did not find any seropositive psychosis cases, or found equivalent prevalence in healthy controls. One study found similar seropositivity for LGI1 and CASPR2 antibodies in patients with established schizophrenia and in healthy controls. Another found no antibodies against AMPAR in 459 participants with psychiatric illness or healthy controls. No studies have examined the prevalence of GABA, R antibodies in patients with psychosis.

For these encephalitides, early treatment with first-line (eg, steroids, plasmapheresis, or intravenous immunoglobulins) or second-line (eg, rituximab or cyclophosphamide) immunotherapy is generally associated with good outcome, and many patients are able to return to a premorbid status.^{9,10} Recognition that these encephalitic syndromes can cause psychosis before development of other symptoms has led to considerable interest as to whether these IgG autoantibodies are associated with psychotic symptoms without the emergence of other features of encephalitis in patients presenting to psychiatric services.

In 2010, we identified serum antibodies to the NMDAR or VGKC-complex in three (6.5%) of 46 patients presenting to a first-episode psychosis service.¹¹ A systematic review and meta-analysis12 showed an odds ratio (OR) of 3.1 (95% CI 1.04-9.27) for serum NMDAR IgG antibody positivity in patients with schizophrenia or schizoaffective disorder, bipolar affective disorder, or major depressive disorder compared with controls, and a later study¹³ of serum NMDAR antibodies in children has supported this finding. However, these studies have considerable heterogeneity in terms of diagnosis, duration of illness, and assay method used for detection of antibodies, and some large case-control studies14-16 have found no difference between patients with psychosis and controls in rates of NMDAR, LGI1, or CASPR216 antibodies in serum. These studies also did not distinguish between antibody subtype (IgG, A, or M).

Added value of this study

We found no overall differences in prevalence of antibodies against neuronal cell surface proteins in the serum of well characterised patients with first-episode psychosis and with less than 6 weeks on antipsychotic drugs compared with a healthy control sample. NMDAR antibodies were present in the serum samples of patients with first-episode psychosis whereas none were detected in controls. This result provides an improved estimate of the prevalence of neuronal cell surface antibodies associated with the onset of psychotic illness.

Implications of all the available evidence

Our finding that NMDAR antibodies can be detected in the serum of patients with first-episode psychosis at a higher rate than in controls supports existing evidence that reduced activity of the NMDAR has an important role in schizophrenia. Antibodies to the NMDAR at the onset of illness might be the basis for these findings in some patients. Patients with these antibodies might respond to treatment with immunotherapy and we therefore suggest screening of patients with firstepisode psychosis.

 $\label{eq:NMDA=N-methyl-D-aspartate receptor. LG11=leucine-rich glioma inactivated 1. CASPR2=contactin associated protein 2. AMPAR=a-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor. GABA_R=\gamma-aminobutyric-acid receptor.$

In this study we aimed to provide an improved estimate of the prevalence of NMDAR and other disease-relevant neuronal cell surface antibodies in the serum of patients with first-episode psychosis, compared with a group of healthy controls with similar age, sex, and ethnicity characteristics. Given the association between autoantibody-mediated CNS disease and cognitive impairment,17,18 and to investigate further the possibility that autoantibody positivity might delineate a distinct phenotypic subgroup of patients with psychosis, we also aimed to characterise the clinical and cognitive profile of our participants. We measured antibodies in serum rather than in cerebrospinal fluid (CSF) both to align with clinical practice in the UK, and because antibodies are detected at a higher rate in serum than in CSF for encephalitis, especially early in the course of illness.²

Although some evidence suggests that non-IgG antibodies (ie, IgM, IgA) might have pathogenic potential within the CNS,¹⁹ these antibodies are very uncommonly detected with live cell assays (AV, LJ; unpublished data) and the study was restricted to IgG antibodies.

Methods

Study design and participants

For identification of cases, 35 early intervention, community, or inpatient mental health services sites across England recruited patients between Feb 1, 2013,

and Aug 31, 2014, experiencing a first episode of psychosis. Inclusion criteria comprised age 14–35 years inclusive, less than 6 weeks on antipsychotic medication, and a score of 4 or more on at least one of the following Positive and Negative Syndrome Scale (PANSS)²⁰ positive items: delusions, hallucinations, grandiosity, or suspiciousness, or on unusual thought content (PANSS general item). Exclusion criteria were suspected drug-induced psychosis or the presence of neurological disorder (eg, head injury, multiple sclerosis). The East of England (Norfolk) Local Research Ethics Committee approved the patient study, reference 12/EE/0307.

Control participants were recruited from the general population in Cambridge, UK as part of a separate study (Local Research Ethics Committee reference 08/H0308/5).²¹ The number of controls was therefore limited by the original study. Inclusion criteria included age older than 16 years, with no personal or family history of mental illness. The control sample was similar in age, sex, and ethnicity to a typical sample of patients with first-episode psychosis. Written informed consent was obtained from every participant to use samples in future studies.

Procedures

Patients were assessed by research assistants local to the centres at a single baseline assessment session with the following clinical measures: psychotic symptoms (PANSS²⁰ with positive, negative, and general psychopathology symptom sub-scores); general level of functioning for people with psychiatric disorders (Global Assessment of Functioning [GAF]);²² catatonic

	Patients with first- episode psychosis (n=228)	Control group (n=105)	p value
Age (years)			
Mean (SD)	24·3 (4·64)	23.8 (4.55)	0.36*
Median (range)	24.0 (16-35)		
Sex			
Male	141 (62%)	67 (64%)	0.77
Female	86 (38%)	38 (36%)	
Ethnic origin			
White	181 (79%)	90 (86%)	0.19
Asian	19 (8%)	2 (2%)	
Black	16 (7%)	10 (10%)	
Other	11 (5%)	3 (3%)	
Alcohol users	105 (46%)	82 (78%)	<0.0001
Cigarette smokers	120 (53%)	16 (15%)	<0.0001
Illicit drug users	57 (25%)	14 (13%)	0.0149

Data are n (%) unless otherwise specified. p values are calculated by χ^2 test unless otherwise specified. Ethnicity was compared using a single $4 \times 2 \chi^2$ test. Clinical data was not provided for one patient. *t test.

Table 1: Characteristics of patients with first-episode psychosis and control participants

symptoms (Bush-Francis Catatonia Rating Scale);²³ and brief cognitive assessment (Addenbrooke's Cognitive Examination-III [ACE-III]).²⁴

We assessed PANSS rating concordance by asking research assistants involved in data collection to rate a standardised video of a PANSS interview. Inter-rater reliability was good, with each research assistant score within 1 SD of the mean. 6-month outcomes were assessed from notes by treating clinicians through a case report form at 6 months, including GAF and illness course.

All patients and controls gave a venous blood sample; patient samples were taken at initial clinical assessment, and control samples were taken after consent in the original study.²¹

BL, LJ, HF, BF, and AV did autoantibody testing in the neuroimmunology laboratory at John Radcliffe Hospital (Oxford, UK) using assays in routine clinical use.^{2,25} We used a live cell-based assay for the detection of IgG antibodies binding to the NMDAR NR1/NR2b subunits, the VGKC-complex-associated proteins LG11 and CASPR2, α1 and γ2 subunits of GABA_AR, and AMPAR, as described elsewhere.^{3,5,25} Binding to the cell membrane was scored by fluorescence microscopy, with a visual score ranging from 0 to 4.² The titre of each antibody was given as the dilution of serum providing a score of 1. All assays were repeated and checked for IgG specificity—as per routine diagnosis and scored separately and masked to diagnosis on each occasion.

We measured VGKC-complex antibodies using a radioimmunoprecipitation assay of VGKC-complex proteins labelled with ¹²⁵I- α -dendrotoxin and precipitated with patient serum samples.²⁵ We measured antinuclear antibodies by direct immunofluorescence as an additional test of autoimmunity.

Statistical analysis

We calculated a sample size of 235 patients to replicate the prevalence of 6.5% found in our pilot study¹¹ while refining the OR estimation to be greater than 5%, thus providing a closer estimation of the prevalence of the disorder. We used *t* tests to compare continuous data and χ^2 tests to compare categorical demographic and lifestyle variables in patients with first-episode psychosis and controls. We used χ^2 tests and ORs (unadjusted and adjusted for smoking, alcohol, and illicit drug use) to compare the prevalence of antibodies in patients and controls. We used likelihood ratios (unadjusted) to compare the prevalence of NMDAR and LG11, because ORs could not be calculated owing to null values in the control group.

We used χ^2 tests and *t* tests (or Mann-Whitney *U* tests with non-normally distributed data) to test for associations between clinical and cognitive test variables and antibody status in patients with first-episode psychosis. We set significance level for all analyses at p=0.05. We analysed data using SPSS version 22.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Feb 1, 2013, and Aug 31, 2014, 235 patients with first-episode psychosis consented to be included in the study. Two patients were excluded after assessment as they did not meet the inclusion criteria, and five patients withdrew their data and samples. 228 patients had serum collected and were included in the study for analysis. These patients and 105 healthy controls were similar in age, sex, and ethnic origin (table 1). The youngest patient was aged 16 years. Patients were more likely to be current cigarette smokers or current users of illicit drugs than were controls, whereas control participants were more likely to be current alcohol drinkers (defined as having consumed alcohol in the previous month).

On antibody testing, 20 (9%) patients with first-episode psychosis were positive for any one neuronal cell surface antibody compared with four (4%) controls (table 2), but this difference was not significant. Serum NMDAR antibodies were more prevalent in patients (seven cases [3%]) than in controls (none). VGKC-complex antibodies were present in 11 (5%) patients and three (3%) controls (p=0.38). One patient was positive for both NMDAR and VGKC-complex antibodies. Patients and controls did not differ in the presence of serum antibodies against LGI1, CASPR2, or GABAAR. No patients or controls had AMPAR antibodies. Overall, titres of participants with positive antibodies were low. No participants with VGKCcomplex antibodies had antibodies against LGI1 or CASPR2. No patients with neuronal antibodies had antinuclear antibodies, and no significant difference was found in antinuclear antibodies between the two populations (table 2). Because the patient and control groups differed in rates of alcohol use, cigarette smoking, and illicit drug use, adjusted ORs were calculated and group differences remained non-significant.

For patients with first-episode psychosis, the neuronal cell surface antibody-positive (n=20) and antibodynegative (n=208) groups were compared on assessments of clinical and cognitive symptoms (table 3). Both groups had levels of psychotic symptoms that indicated a moderate level of illness (PANSS total $69 \cdot 0-77 \cdot 6$), with antibody-positive patients having lower mean PANSS positive scores and mean PANSS total scores than did antibody-negative patients (table 3). Both groups had low levels of catatonia symptoms, with antibody-positive patients having lower mean total scores than did antibody-negative patients. Both antibody-positive and antibody-negative patients had impairment in their cognitive functioning and were moderately functionally impaired with mean GAF scores of around 50, but with no significant differences in these parameters between groups (table 3).

Patients positive for autoantibodies were followed up 6 months after baseline assessment to investigate clinical course and outcome. We found no significant difference in the number of increased contacts with mental health services (either hospital admission or treatment with a home treatment or crisis service) between the antibody-positive patients (mean 0.4, SD 0.6) and the antibody-negative patients (0.5, 0.7; p=0.51), or in the mean follow-up GAF ratings (antibody-positive 66.5 [SD 15.5] *vs* antibody-negative 63.4 [15.7]; p=0.52). No cases of encephalitis or development of neurological symptoms were reported in any patients, as assessed by their treating psychiatrist.

Discussion

In this study, which is the largest to our knowledge to examine the prevalence of neuronal cell surface antibodies in serum samples of patients with first-episode psychosis, we have shown that NMDAR antibodies were more prevalent in patients with first-episode psychosis than in the healthy control group. The prevalence of antibodies against GABAAR, LGI1, and VGKC-complex did not differ between the groups. ORs adjusted for current smoking, alcohol consumption, and illicit drug use were also non-significant. No major differences were found in clinical phenotype between antibody-positive and antibody-negative cases; however, patients who tested positive for neuronal cell surface antibodies scored significantly lower on PANSS positive items and PANSS total score, as well as on the Bush-Francis Catatonia Rating Scale.

Our NMDAR antibody findings corroborate an earlier report¹¹ that found that two (4%) out of 46 patients with

	Titres	Patients with first-episode psychosis (n=228)	Controls (n=105)	Odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
NMDAR antibodies	1:30-1:150	7 (3%)	0	5·4 (p=0·0204)†	
LGI1 antibodies	1:20-1:100	3 (1%)	0	2·3 (p=0·1298)†	
CASPR2 antibodies	1:100-1:250	2 (1%)	3 (3%)	0.3 (0.1–1.8)	2.2 (0.3–17.1)
GABA _A Rantibodies	1:50-1:100	8 (4%)	1 (1%)	3.8 (0.5-30.7)	0.4 (0.3–3.6)
AMPAR antibodies		0	0		
Any neuronal cell surface antibody		20 (9%)	4 (4%)	2.4 (0.8–7.3)	0.5 (0.1–1.7)
Other antibodies					
VGKC-complex antibodies >150 pM‡		11 (5%)	3 (3%)	1.7 (0.5–6.3)	0.8 (0.2–3.2)
Antinuclear antibodies >1/160		7 (3%)	9 (9%)	0.5 (0.2–1.4)	3.6 (1.0–13.6)

NMDAR=N-methyl-D-aspartate receptor. LGI1=leucine-rich glioma inactivated 1. CASPR2=contactin associated protein 2. GABA, R= γ -aminobutyric-acid receptor. AMPAR= α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor. VGKC=voltage-gated potassium channel. *Odds ratio adjusted for current smoking, alcohol, and illicit drug use. †Likelihood ratio (p value). ‡Measured by radioimmunoassay.

Table 2: Prevalence of neuronal cell surface antibodies in patients and controls

	Antibody- positive patients (n=20)	Antibody- negative patients (n=208)	p value				
Clinical symptoms and functioning							
PANSS							
Positive symptom score	19·1 (3·7)	21.8 (6.1)	0.0087*				
Negative symptom score	15·2 (7·8)	17.0 (6.7)	0.29*				
Global score	34.8 (7.9)	38.8 (10.3)	0.11*				
Total score	69·0 (17·2)	77.6 (17.8)	0.0508*				
ACE-III							
Attention	16.4 (1.8)	16.6 (2.0)	0.75*				
Fluency	9.5 (2.4)	9.9 (3.0)	0.61*				
Language	22.1 (3.8)	22.4 (3.8)	0.69*				
Memory	19.1 (5.7)	19.4 (5.7)	0.83*				
Visuospatial	14.7 (1.8)	14.4 (2.2)	0.49*				
Total	81.8 (10.8)	81.8 (14.7)	0.99*				
Catatonia Rating Scale	0.6 (1.1)	2.2 (3.7)	0.0002*				
GAF	54.2 (16.1)	48.9 (15.7)	0.18*				
Illness history and family history							
Estimated DUP (months)	2.1 (3.3)	7.0 (25.5)	0.93†				
Median DUP (months)	1.1	0.8					
Prodromal symptoms							
Attenuated psychosis or blip	4 (20%)	42 (20%)	0.92‡				
Low mood or anxiety§	9 (45%)	66 (32%)	0.17‡				
Physical (headache, viral illness)§	5 (25%)	52 (25%)	0.90‡				
Family history							
Psychiatric	9 (45%)	110 (53%)	0.65‡				
Autoimmune	1 (5%)	11 (5%)	0.99‡				

Family history is any psychiatric or autoimmune disorder in a first-degree relative. Data are mean (SD) or n (%) unless otherwise indicated. PANSS=Positive and Negative Syndrome Scale. ACE-III=Addenbrooke's Cognitive Examination-III. GAF=Global Assessment of Functioning. DUP=duration of untreated psychosis. *t test. †Mann-Whitney U test (data not normally distributed). $\ddagger\chi^2$ test. \$Some patients had more than one prodromal symptom and have been counted as positive in each category.

Table 3: Comparison of antibody-positive and antibody-negative patients with first-episode psychosis on baseline clinical measures

first-episode psychosis had NMDAR antibodies, and provide support for the many converging lines of research evidence suggesting that NMDAR hypofunction plays an important part in schizophrenia.²⁶ Susceptibility genes for schizophrenia—both common and rare variants—are particularly associated with glutamatergic transmission and with the adaptive immune system.²⁷ Pharmacological blockade of NMDAR produces the full spectrum of symptoms seen in schizophrenia, as well as the neuropathological findings of reduced numbers of inhibitory GABAergic interneurons and reduced dendritic spine density that are compatible with a model of NMDAR blockade.^{26,28} Antibodies to the NMDAR at the onset of illness might be the basis for these findings in some patients.

We used a live cell-based assay to assess antibody status for all antibodies except VGKC-complex and antinuclear antibodies. In a live cell-based assay, the serum to be tested for autoreactivity is incubated with live HEK293 cells-previously transfected with plasmids encoding the specific antigen subunits-before these cells are fixed.² By contrast, both commercial assays and those performed by most other laboratories involve fixation or permeabilisation of cells before incubation with serum or CSF.29 The effects of fixation and permeabilisation (and other inter-assay differences) on the relative sensitivities and specificities of these assays are unknown. This situation is particularly true when the assay is used in psychiatric populations for whom-unlike in some autoimmune CNS disorders such as neuromyelitis optica³⁰—no well established and clinically defined group exists against which such sensitivity and specificity can be assessed independently of autoantibody status. However, in assays other than live cell-based assays, permeabilisation of the cell membrane probably exposes intracellular antigens, and so seropositivity might indicate the presence of antibodies that can bind intracellular epitopes; these antibodies would not be expected to be pathogenic.

VGKC-complex antibody levels, as measured by radioimmunoassay, did not differ significantly between patients with first-episode psychosis and controls, and no individuals with VGKC-complex antibodies had antibodies against LGI1 or CASPR2. The relevance of these antibodies in psychosis is therefore uncertain, and a high level of VGKC-complex antibodies alone might not be clinically relevant in psychosis.

In this study, antibody-positive patients had a lower level of catatonic and psychotic symptoms than did antibody-negative patients. The absolute difference in symptom scores was modest, and does not reflect clinically meaningful differences. However, this result contrasts with a previous case series of patients with NMDAR antibodies and psychosis in which the patients were described as being more psychiatrically unwell than a typical group of patients with schizophrenia, with increased catatonia and cognitive impairment, and with adverse reactions to antipsychotics.³¹ These previous cases might have been subject to selection bias, whereby clinicians preferentially requested the antibody test only if the patient presented with atypical features suggestive of encephalitis.

The prevalence of IgG antibodies detected in this study is higher than that described in most other groups of patients with either first-episode psychosis or longstanding illness (ranging from 0.0% to 1.6%).¹⁴⁻¹⁶ A notable exception is a study¹³ in children with psychosis tested a median of 5 weeks after onset of symptoms, which found 12% prevalence of NMDAR IgG antibodies, with none in healthy or illness (neurological or general medical) controls. A possible explanation is the short length of

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treatment with antipsychotics in our group. Antipsychotics have been shown to have immunomodulatory properties,³² even showing therapeutic promise in an animal model of autoimmune encephalitis.³³ Some studies^{13,15,16} have detected IgM and IgA antibodies to neuronal targets, but we did not specifically look for IgM or IgA antibodies in this study as previous attempts to demonstrate them on live NMDAR assays have been unsuccessful (AV, LJ; unpublished data).

A limitation of the study is that we did not collect CSF, owing to the impracticality of doing lumbar punctures in routine UK mental health settings. Detection of antibodies in CSF might provide a more definitive indicator of the functional relevance of the antibodies. However, a study³⁴ has shown that antibodies that have crossed the blood-brain barrier tend to bind to the brain-with the brain acting as an immunoprecipitatorand will therefore not be measurable in CSF. Therefore, an absence of antibodies in CSF does not prove that antibodies are not causing illness in an individual. A further limitation to our study is that antibody status was measured at a single timepoint only. We were therefore unable to establish whether autoreactivity precedes the development of symptoms, which is the case in many autoimmune diseases. If so, a second hit causing disruption of the blood-brain barrier could plausibly be required for pathogenic antibodies to access the CNS and affect neuronal function, as has been suggested elsewhere.¹⁶ We looked only at patients with first-episode psychosis. Pathogenic antibodies might be associated with other acute or subacute onset neuropsychiatric disorders with overlapping features with encephalitis, such as depression, dementia, or acute confusional states.

Further work is required to establish the specificity and pathogenicity of antibodies in the context of psychosis. The demonstration that even a small proportion of cases of schizophrenia-like psychosis have an autoimmune basis would have important ramifications for nosology and for treatment, because affected patients might respond to immunotherapy rather than antipsychotics or psychological interventions. Our study suggests that, at present, the only way to detect patients with these potentially pathogenic antibodies is to screen all patients with first-episode psychosis at first presentation.

Contributors

BRL, PBJ, PJH, and AV contributed to the initial study design. JH, ECP-C, and the PPiP study group contributed to patient data collection. LS did the control sample collection. LJ, BL, HF, BF, and AV were responsible for the antibody assays and sample analysis. BRL, ECP-C, TP, JH, JM, and HC contributed to data analysis. All authors contributed to the manuscript and gave final approval of the version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declaration of interests

AV and the University of Oxford hold patents for antibody assays, and AV receives a proportion of royalties from Athena Diagnostics and Euroimmun AG. BL has a patent for the use of LG11 in diagnosis of autoantibody-mediated disease. All remaining authors declare no competing interests.

References

- 1 Dalmau J, Tüzün E, Wu H, et al. Paraneoplastic anti-N-methyl-Daspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007; 61: 25–36.
- 2 Irani SR, Bera K, Waters P, et al. N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. *Brain* 2010; 133: 1655–67.
- B Irani SR, Alexander S, Waters P, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 2010; 133: 2734–48.
- Bataller L, Galiano R, García-Escrig M, et al. Reversible paraneoplastic limbic encephalitis associated with antibodies to the AMPA receptor. *Neurology* 2010; 74: 265–67.
- 5 Pettingill P, Kramer HB, Coebergh JA, et al. Antibodies to GABAA receptor $\alpha 1$ and $\gamma 2$ subunits: clinical and serologic characterization. *Neurology* 2015; 84: 1233–41.
- 6 Graus F, Boronat A, Xifró X, et al. The expanding clinical profile of anti-AMPA receptor encephalitis. *Neurology* 2010; 74: 857–59.
- 7 van Sonderen A, Schreurs MWJ, de Bruijn MAAM, et al. The relevance of VGKC positivity in the absence of LGI1 and Caspr2 antibodies. *Neurology* 2016; 86: 1692–99.
- 8 Hacohen Y, Singh R, Rossi M, et al. Clinical relevance of voltage-gated potassium channel–complex antibodies in children. *Neurology* 2015; 85: 967–75.
- 9 Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol* 2013; 12: 157–65.
- 10 Irani SR, Stagg CJ, Schott JM, et al. Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain* 2013; 136: 51–62.
- 11 Zandi MS, Irani SR, Lang B, et al. Disease-relevant autoantibodies in first episode schizophrenia. J Neurol 2011; **258**: 686–88.
- 12 Pearlman DM, Najjar S. Meta-analysis of the association between N-methyl-d-aspartate receptor antibodies and schizophrenia, schizoaffective disorder, bipolar disorder, and major depressive disorder. *Schizophr Res* 2014; 157: 249–58.

- 13 Pathmanandavel K, Starling J, Merheb V, et al. Antibodies to surface dopamine-2 receptor and N-methyl-D-aspartate receptor in the first episode of acute psychosis in children. *Biol Psychiatry* 2015; 77: 537–47.
- 14 de Witte LD, Hoffmann C, van Mierlo HC, Titulaer MJ, Kahn RS, Martinez-Martinez P. Absence of N-methyl-d-aspartate receptor IgG autoantibodies in schizophrenia: the importance of cross-validation studies. *JAMA Psychiatry* 2015; 72: 731–33.
- 15 Dahm L, Ott C, Steiner J, Stepniak B, et al. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann Neurol* 2014; 76: 82–94.
- 16 Steiner J, Walter M, Glanz W, et al. 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. JAMA Psychiatry 2013; 70: 271–78.
- 17 Finke C, Kopp UA, Prüss H, Dalmau J, Wandinger K-P, Ploner CJ. Cognitive deficits following anti-NMDA receptor encephalitis. J Neurol Neurosurg Psychiatry 2012; 83: 195–98.
- 18 Butler CR, Miller TD, Kaur MS, et al. Persistent anterograde amnesia following limbic encephalitis associated with antibodies to the voltage-gated potassium channel complex. *J Neurol Neurosurg Psychiatry* 2014; 85: 387–91.
- 19 Castillo-Gómez E, Oliveira B, Tapken D, et al. All naturally occurring autoantibodies against the NMDA receptor subunit NR1 have pathogenic potential irrespective of epitope and immunoglobulin class. *Mol Psychiatry* 2016; published online Aug 9. DOI:10.1038/mp.2016.125.
- 20 Kay SR, Flszbein A, Opfer LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 1987; 13: 261.
- 21 Scoriels L, Salek RM, Goodby E, et al. Behavioural and molecular endophenotypes in psychotic disorders reveal heritable abnormalities in glutamatergic neurotransmission. *Transl Psychiatry* 2015; 5: e540.
- 22 American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-4. Washington, DC: American Psychiatric Association, 1994.
- 23 Bush G, Fink M, Petrides G, Dowling F, Francis A. Catatonia. I. Rating scale and standardized examination. *Acta Psychiatr Scand* 1996; 93: 129–36.

- 24 Hsieh S, Schubert S, Hoon C, Mioshi E, Hodges JR. Validation of the Addenbrooke's Cognitive Examination III in frontotemporal dementia and Alzheimer's disease. *Dement Geriatr Cogn Disord* 2013; 36: 242–50.
- 25 Paterson RW, Zandi MS, Armstrong R, Vincent A, Schott JM. Clinical relevance of positive voltage-gated potassium channel (VGKC)-complex antibodies: experience from a tertiary referral centre. J Neurol Neurosurg Psychiatry 2014; 85: 625.
- 26 Coyle J. NMDA receptor and schizophrenia: a brief history. Schizophr Bull 2012; 38: 920–26.
- 27 Harrison PJ. Recent genetic findings in schizophrenia and their therapeutic relevance. *J Psychopharmacol* 2015; **29**: 85–96.
- 28 Zhang Y, Behrens MM, Lisman JE. Prolonged exposure to NMDAR antagonist suppresses inhibitory synaptic transmission in prefrontal cortex. J Neurophysiol 2008; 100: 959–65.
- 29 Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol* 2008; 7: 1091–98.
- 30 Waters P, Reindl M, Saiz A, et al. Multicentre comparison of a diagnostic assay: aquaporin-4 antibodies in neuromyelitis optica. J Neurol Neurosurg Psychiatry 2016; 87: 1005–15.
- 31 Zandi MS, Deakin JB, Morris K, et al. Immunotherapy for patients with acute psychosis and serum N-methyl-D-aspartate receptor (NMDAR) antibodies: a description of a treated case series. *Schizophr Res* 2014; 160: 193–95.
- 32 MacDowell KS, García-Bueno B, Madrigal JLM, et al. Risperidone normalizes increased inflammatory parameters and restores anti-inflammatory pathways in a model of neuroinflammation. *Int J Neuropsychopharmacol* 2013; 16: 121–35.
- 33 O'Sullivan D, Green L, Stone S, et al. Treatment with the antipsychotic agent, risperidone, reduces disease severity in experimental autoimmune encephalomyelitis. *PLoS One* 2014; 9: e104430.
- 34 Castillo-Gomez E, Kästner A, Steiner J, et al. The brain as immunoprecipitator of serum autoantibodies against N-methyl-D-aspartate receptor subunit NR1. Ann Neurol 2016; 79: 144–51.