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Brain-relevant antibodies in first-episode psychosis: a matched case-control study.

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Brain-relevant antibodies in First Episode Psychosis: A matched case-control study

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Disclosure

FG has received honoraria for advisory work and lectures from Roche, BMS, Lundbeck, Otsuka and Sunovion, is a collaborator on a NHS Innovations project co-funded by Janssen and has a family member with professional links to Lilly and GSK, including stock

RSF is now an employee of Otsuka Pharmaceuticals.

EP is an employee of Mundipharma Research Limited, Cambridge, UK

RMM has received payment for lectures including service on speakers' bureaus for BMS, Janssen, AZ.

AV and the University of Oxford hold patents and receive royalties for antibody tests including LGI1 and CASPR2.

The other authors, JL, KB, RM, PGS, EC, LJ, BL, MF and TN have no conflict of interest to declare.

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Abstract

Background

There has been much recent excitement about the possibility that some cases of psychosis may be wholly due to brain-reactive antibodies, with antibodies to N-Methyl-D-Aspartate receptor (NMDAR) and the voltage gated potassium channel (VGKC)-complex reported in a few patients with first episode psychosis (FEP).

Methods

Participants were recruited from psychiatric services in South London, UK, from 2009-2011 as part of the Genetics and Psychosis (GAP) study.

We conducted a case control study to examine NMDAR and VGKC-complex antibody levels and rates of antibody positivity in ninety-six patients presenting with FEP and ninety-eight controls matched for age and sex. LGI1 and CASPR antibodies were also measured. Notably, patients with suspicion of organic disease were excluded.

Results

VGKC-complex antibodies were found in both cases (n=3) and controls (n=2). NMDAR antibody positivity was seen in one case and one control. Either LGI1-Abs or CASPR2-Abs were found in three cases and three controls. Neuronal antibody staining, consistent with the above results or indicating potential novel antigens, was overall positive in four patients but also in six controls. Overall, antibody positivity was at low levels only and not higher in cases than in controls.

Conclusions

This case-control study of the prevalence of antibodies in FEP does not provide evidence to support the hypothesis that FEP is associated with an immune-mediated process in a subgroup of patients. Nevertheless, as other bio-clinical factors may influence the effect of such antibodies in a given individual, and patients with organic neurological disease may be misdiagnosed as FEP, the field requires more research to put these findings in context.

Keywords: First episode psychosis; FEP; NMDA; VGKC-complex; schizophrenia; antibodies

Title:

Brain-relevant antibodies in First Episode Psychosis: A matched case-control study

Introduction

The link between psychosis and autoimmunity has been explored over many decades (Benros *et al.*, 2014, Eaton *et al.*, 2006, Wright *et al.*, 1999), but only in recent years has this been related to the pathophysiology of psychosis at a neurotransmitter level via specific antibodies against antigens which have clearly established links to the aetiology of psychosis. This has resulted in a surge of interest in whether a sub-group of people with psychosis have serum antibodies to certain neuronal antigens, although the specificity, sensitivity and clinical relevance of these remain unclear (Coutinho *et al.*, 2014, Deakin *et al.*, 2014, Pollak *et al.*, 2014). These auto-antibodies target synaptic or extra-synaptic membrane-expressed proteins, with greatest interest in antibodies to the N-Methyl-D-Aspartate receptor (NMDAR) and the voltage gated potassium channel (VGKC)-complex, which includes antigens leucine- rich glioma inactivated-1(LGII) and contactin-associated protein-2 (CASPR2) (Ezeoke *et al.*, 2013, Pearlman and Najjar, 2014, Steiner *et al.*, 2013, Zandi *et al.*, 2011).

Psychotic symptoms are a common feature of the encephalitis syndromes classically associated with such antibodies – often occurring before the development of other features of encephalitis, such as seizures, movement disorders, autonomic instability and impaired consciousness. For example, in the seminal case series describing 100 anti-NMDAR encephalitis cases, the majority (77%) of patients initially presented to psychiatry services with predominantly psychotic symptoms (Dalmau *et al.*, 2008). A subsequent larger case series of 571 patients reported 18 patients (3.2 %) having an ‘isolated’ psychiatric relapse (after an initial ‘full’ encephalitis). Five further patients (0.9 %) had ‘isolated’ psychiatric symptoms, again predominantly psychosis, at presentation – although on retrospective close examination they were found to have had other clinical features and were re-diagnosed as encephalitis (Kayser *et al.*, 2013). The key question is therefore whether isolated psychosis, i.e. schizophrenia, is associated with these antibodies.

A prospective cohort study in first episode psychosis (FEP) identified serum NMDAR antibodies in 6.4% of patients (n=3/47) (Zandi *et al.*, 2011) using a live cell-based assay (CBA) and well-established subjective visual scoring system (Irani *et al.*, 2010b). A recent case-control study in FEP identified an increased prevalence of NMDAR antibodies (n=7/228; 3% of total population) compared to controls (n=0/105), with no difference in the prevalence of LGI1, CASPR2 or VGKC-complex antibodies between the groups (Lennox *et al.*, 2017). Using the same methodology, a recent study by our group found that 7% of patients (n=3/43) with chronic refractory psychosis were positive for NMDAR antibodies (Beck *et al.*, 2015). Methodology, however, appears to be relevant, as 9.9% of patients with acute schizophrenia were reported NMDAR antibody positive using the commercial fixed CBA, with similar rates in controls (Steiner *et al.*, 2013), whereas, other studies have failed to identify any antibody positivity in either FEP (Masdeu *et al.*, 2012) or established schizophrenia (de Witte *et al.*, 2015, Haussleiter *et al.*, 2012, Rhoads *et al.*, 2011). Some of these negative studies also used fixed CBA to measure NMDAR seropositivity, but there have been few direct comparisons between laboratories (Wandinger *et al.*, 2011). A meta-analysis identified no excess of NMDAR antibody (of any subtype (IgG, IgM, IgA)) in psychotic disorders, though IgG antibodies alone were significantly greater in FEP cases (IgG positive in 5/272 of cases) than in controls (IgG positive in 5/1598 of controls) (Pollak *et al.*, 2014).

VGKC-complex antibodies can also cause cell-surface antibody associated CNS disorders (Zuliani *et al.*, 2012). Most of these antibodies bind to LGI-1 or CASPR-2 (Irani *et al.*, 2010a), which are cell surface proteins that form part of the VGKC-complex. The targets of other VGKC-complex antibodies are unclear, although two recent studies suggest that they bind to intracellular epitopes and may be neuroinflammatory biomarkers rather than pathogenic antibodies (Hacohen *et al.*, 2015, Lang *et al.*, 2017).

Many patients with high levels of VGKC-complex antibodies have evidence of limbic encephalitis and some present first with psychosis (Paterson *et al.*, 2014). Phenotypically, LGI1 antibodies tend to associate with limbic encephalitis and faciobrachial dystonic seizures, phenomena which can be misinterpreted as myoclonic jerks (Irani *et al.*, 2011). CASPR2 antibodies, meanwhile, are associated

with neuromyotonia or Morvan's syndrome in which psychiatric features are common, often with a thymoma (Irani *et al.*, 2010a). In a case series, over a third of patients with VGKC-complex antibodies had psychiatric symptoms, including confusion, personality change, memory impairment and depression (Somers *et al.*, 2011). Those affected are classically older than those with NMDAR antibody encephalitis. VGKC-complex antibodies were identified in one patient out of 46 with FEP (Zandi *et al.*, 2011).

The importance of antibodies to NMDAR and VGKC-complex proteins is clear when psychosis presents in the context of encephalitis, but the challenge in psychiatry is to determine their relevance in clinically uncomplicated psychosis. Only one study to date has compared the prevalence of both these, and other neuronal antibodies in adults with FEP compared to matched controls (Lennox *et al.*, 2017).

Methods and Materials

Participants

The study population comprised 96 adults with a FEP and 98 matched controls. The participants were recruited as part of the Genetics and Psychosis (GAP) study. The GAP study is a case-control study investigating the genetic basis of susceptibility to psychosis.

Participants were recruited from both inpatient and outpatient settings through working closely with the clinical teams. Patients were consented as soon after first presentation with psychosis as possible (the mean duration from the time of first contact for psychosis to the time of blood sampling was 52.74 (SD=37.3) days (median 47 days ; range 7-167 days)). Assessments were performed by trained research workers and Operational Criteria Checklists (OPCRIT) diagnoses made by research clinicians.

Participants were aged 18-65 years. Patients were eligible if they had a first episode psychosis and met the ICD-10 criteria for psychosis (ICD-10 codes F20-29 and F30-33) diagnosed utilising the OPCRIT (McGuffin *et al.*, 1991). The baseline diagnoses were made from face-to-face interviews by trained researchers and mental health records according to ICD-10 criteria (WHO, 1992) utilising the OPCRIT

(McGuffin *et al.*, 1991). Participants had made first contact with our health services for psychotic symptoms not more than 12 months previously.

Study Design

Patients were matched for gender and age (plus or minus 5 years) to 98 healthy controls from the same local population, recruited by means of internet and newspaper advertisements, and distribution of leaflets at train stations, shops and job centres. Those who agreed to participate as controls were administered the Psychosis Screening Questionnaire (Bebbington and Nayani, 1995) and excluded if they met criteria for a psychotic disorder or reported a previous diagnosis of psychotic illness.

Exclusion criteria for both controls and cases were an organic psychosis (ICD-10: F06.8 and F9.0), i.e a psychosis deemed likely to be secondary to neurological disorders such as epilepsy, head injury, or to a long term deterioration of intellectual function and memory (dementia) or a short term disturbance of orientation, judgement, or consciousness (delirium). The Schedule for Clinical Assessment in Neuropsychiatry (SCAN) was administered to elucidate psychotic symptoms present in the month before study recruitment, from which those cases meeting criteria for organic psychosis were excluded. These decisions were made in discussion with two experienced psychiatrists. Those with a substance induced psychosis as defined by ICD-10, a diagnosis of intellectual disability, terminal cancer, and pregnancy were also excluded.

All participants provided written informed consent. The project was approved by the Research Ethics Committee of the Joint South London and Maudsley and The Institute of Psychiatry NHS Research Ethics Committee.

Specimen Collection

The samples were transferred to Oxford for testing in two batches during the course of the observational study. The first group consisted of plasma from 30 patients and 30 age and sex matched controls while the second group was serum from 66 patients and 68 controls matched for age and sex.

Plasma samples

Samples were collected in EDTA tubes using a 23 gauge butterfly needle and vacutainer needle holder following standard venepuncture in the cubital region, in the area of anastomosis between the radial and the ulnar veins or in the brachial vein, slightly proximal to this area. To avoid coagulation of the sample, a 9ml K3EDTA tube (Greiner Bio-One, Cat # 455036X - EDTA tube, lavender lid) was used. The sample was immediately kept at +4°C until being processed within two hours. The tube was labelled with the subject barcode with no phenotypic information given and then centrifuged at 3000 rcf (xg), 8 minutes at +4°C; the plasma was aliquoted in 0.5 ml eppendorf tubes and frozen at -80°C within three hours of collection.

Analysis of results for combined plasma and serum samples (n=194), and with serum samples alone are presented (n=134).

Antibody testing

All samples received in Oxford were kept at -20 C until the analyses, blinded to patient-control status, were performed. The antibody assays for NMDAR, LGI1 and CASPR2 were performed using live cell-based assays as established in previous studies (Irani *et al.*, 2010a, Irani *et al.*, 2010b, Zandi *et al.*, 2011) and as used for routine diagnostic testing for the Oxford Neuroimmunology Service. HEK293 cells were transfected with plasmids encoding the human forms of NMDAR (NR1 and NR2B subunits), LGI1 or CASPR2, washed, grown for 24-36 hours and then incubated at room temperature in the plasmas or sera at 1:20 (1:100 for CASPR2 as used routinely for this protein). Binding of antibodies to the transfected antigens on the live cells was detected with Alexa fluor labelled anti-human IgG, and the cells examined under fluorescence microscopy. The binding was compared with known positive and negative controls and scored on a scale from 0 – 4.

Positive results (>0) were repeated and the IgG specificity confirmed with an IgG-specific CBA. This involved replacing anti-human IgG, which can recognise the light chains of IgG, A, M and D, with goat anti-human Fc IgG secondary (Thermo Scientific; 31125; 1:750), followed by mouse anti-goat IgG, Alexa fluor 568 (1:750) for 45 minutes at room temperature (Pettingill *et al.*, 2015). Full details are given in (Pettingill *et al.*, 2015) and in the Appendix (supplementary material 1).

Serum VGKC-complex antibodies were tested using a radio-immunoprecipitation assay (Irani *et al.*, 2010b, Paterson *et al.*, 2014). VGKC results were expressed as picomoles (pM) per litre of serum.

Neuronal cultures were prepared from P0 rat hippocampi and antibody binding performed at 1:100 serum or plasma after 10-14 days in culture (Irani *et al.*, 2010a, Irani *et al.*, 2010b, Pettingill *et al.*, 2015).

Clinical assessments

The degree of psychopathology at first presentation to mental health services was measured on the Positive and Negative Syndrome Scale (PANSS)(Kay *et al.*, 1987). Overall functional disability was assessed using the Global Assessment of Functioning (GAF).

Statistical Analysis

Differences in NMDAR and VGKC-complex antibody prevalence rates in FEP patients and controls were assessed using Chi square and t-tests. All statistical tests were two-sided and the α - level for statistical significance was 0.05.

Results

Sample characteristics

The demographic characteristics of patients with FEP and controls are shown in Table 1.

Cases and controls with positive results are summarized in Table 2. Six percent of cases (n=6/96) and 6% of controls (n=6/98) were positive ($\chi^2=0.001$, $p=0.602$) for specific antibodies (NMDAR, LGI1, CASPR2 or VGKC-complex); of these, three cases and three controls had IgG antibodies binding to hippocampal neurons in culture. An additional one case and three controls had unknown neuronal antibodies only. Overall, nine controls and seven patients had antibody positivity or were positive on neuronal staining, but this difference did not meet statistical significance ($\chi^2=0.229$, $p=0.414$). All

patients with antibody positivity or positive neuronal staining were female (7/47). Five controls with antibody positivity or with neuronal staining were female and 3 were male. Examples of the cell-based assays, neurons and scatter plots of the data are shown in supplementary figure 1.

Of the plasma samples, no antibody positive cases were detected in cases (n=30), but one female control sample was positive for LGI1, with no other antibodies identified in control plasma samples (n=30). When the serum samples alone were considered, therefore, the proportion with antibody positivity or neuronal positivity increased to 8/68 controls (11.8%) and 7/66 patients (10.6%) ($\chi^2=0.045$, $p=0.525$)

NMDAR Antibodies

Standardised immunofluorescent cell based assay (CBA) for the detection of serum IgG antibodies directed against NMDAR was positive in 1% (n=1 female) of cases and 1% (n=1 male) of controls.

VGKC-complex, LGI1 and CASPR2 antibodies

VGKC-complex antibodies were the most prevalent antibody in both cases and controls, found in 3.1% (n=3) of patients and in 2.0% (n=2) of controls ($\chi^2=0.227$, $p=0.490$). In cases, VGKC-complex antibody positivity was found in female patients only (n=3/47) (males (n=0/49) ($\chi^2=3.229$, $p=0.113$)). VGKC-complex antibodies were found in one male and one female control.

Two patients (both female) and two controls (both female) had LGI1 antibodies, though none were associated with VGKC-complex antibodies. CASPR2 antibodies were found in one female patient and in one female control. The patient with CASPR2 antibodies was also positive for NMDAR antibodies.

There were no cases or controls with high positive VGKC-complex antibodies. (>400pM). The mean level in cases was 179.7 (SD 95.8) pM and 212.0 (SD 31.1) pM in controls ($t=0.441$, $p=0.689$). One case with VGKC-complex antibodies (132 pM) and one control (234 pM) also showed neuronal staining.

Associations with clinical measures

There were no significant differences between cases with any antibody positivity and cases which were antibody negative in mean total PANSS score (64.2 (15.4) vs 59.3 (15.1) ($z=0.460$, $p=0.646$); mean PANSS positive scores (18.8 (3.9) vs 15.2(6.0) ($z=1.582$, $p=0.114$); mean PANSS negative score (13.2 (6.6) vs 13.9 (5.3) ($z=-0.607$, $p=0.544$); mean GAF symptom scores (29.6 (12.4) vs 28.9 (12.6) ($z=0.335$, $p=0.737$) or mean GAF disability scores (30.1 (14.7) vs (31.9 (14.4) ($z=-0.502$, $p=0.616$).

Discussion

This is the second matched case-control study to assess the prevalence of antibodies in patients with FEP and matched healthy controls. We did not find evidence to support the hypothesis that FEP is associated with an immune-mediated process in a subgroup of patients. Our findings in healthy adults contrast with those of the recent case control study of 228 FEP patients, in which NMDAR antibodies ($n=7$; 3% of total population) were more prevalent in patients with FEP than in the control group ($n=0/105$) (Lennox *et al.*, 2017). Similar to our study, Lennox *et al* did not identify a significantly increased prevalence of antibodies against LGI1, or VGKC-complex between the groups. Our rates of patient positivity were similar to, and indeed slightly numerically higher than an earlier case series (Zandi *et al.*, 2011), but no higher than in our controls. Our findings therefore fail to support the hypothesis that NMDAR and VGKC-complex antibodies are higher or more prevalent in the sera of patients with FEP than in healthy controls.

This accords with findings in other centres: Steiner *et al.* 2013 found no difference in NMDAR antibody rates between groups in a large cohort of people with acute schizophrenia and controls, although the laboratory methods were different to those used here. A large scale systematic screening did not identify a significant difference in prevalence of NMDAR antibodies between those with schizophrenia (8.6%) and healthy controls (10.8%)(Hammer *et al.*, 2014). In that study, the authors postulated that those with schizophrenia may be rendered vulnerable to the effect of circulating antibodies by previous blood brain barrier perturbation, perhaps due to previous head injury or

infection(Hammer *et al.*, 2014). Likewise, a smaller unmatched case control study found no NMDAR antibodies in either 80 patients with first episode schizophrenia or 40 controls (Masdeu *et al.*, 2012). Our findings in healthy adults contrast with those of a case control study of 43 children with FEP (median age 15 years), which identified NMDAR antibodies in 6 of 43 patients, but in none of the controls (Pathmanandavel *et al.*, 2014).

While we did not find it to be statistically significant, it is intriguing that all 7 patients with antibody or neuronal positivity were female. A predominance of females with NMDAR antibodies is evident in patients with NMDAR-antibody encephalitis with 59% of such cases associated with ovarian tumours (Dalmau *et al.*, 2008). However, subsequent reports tended to find less extreme gender effects (68% female vs 32% males) and lower rates of ovarian tumours (26% of females with NMDAR antibody positivity with ovarian teratoma) (Irani *et al.*, 2010b). Our findings are not consistent with prior studies in established psychosis, in which male patients with antibody positivity prevailed (Steiner *et al.*: 10 male vs 5 female positive; Hammer *et al.*: 11.5% male positive and 8.7% females)(Hammer *et al.*, 2014, Steiner *et al.*, 2013) and there were equivalent prevalence of IgA and IgM antibodies in cases and controls(Hammer *et al.*, 2014). We identified no difference in IgG antibodies, which are considered highly specific for the full NMDAR-antibody encephalitis, between patients and controls.

Our study excluded people presenting with organic psychosis, and so our sample may have fewer patients with neurological/neuropsychiatric features than a less selected group. Nevertheless, if serum disease relevant autoantibodies result in uncomplicated psychosis, one would expect to see an excess of these antibodies in the patient group over controls. We did not, however, test for other factors which may render serum antibodies pathological in patients, such as those affecting blood brain barrier permeability(Hammer *et al.*, 2014), and there is evidence from case series that NMDAR-antibody positive patients with predominant psychosis at onset can be missed or have delayed diagnosis (Barry *et al.*, 2011, Tsutsui *et al.*, 2012).

We restricted our search to IgG antibodies detected on the cell based assays (NMDAR, LGI1, CASPR2) and did not look specifically for other (e.g., IgM, IgA) antibody classes. Although the IgG

antibody subtype was the class that was associated with psychosis in a meta-analysis of NMDAR antibody prevalence in FEP (Pollak *et al.*, 2014), it remains possible that subjects with FEP and controls in this study could differ with respect to other antibody classes. Another potential source of underestimate in antibody prevalence in cases was that only serum or plasma was tested. It has previously been shown that in NMDAR-antibody disease 15% of patients with antibodies in the cerebrospinal fluid (CSF) did not have detectable antibodies in the serum (Gresa-Arribas *et al.*, 2014), although that study used a different method to that used here; nevertheless it would be important in future studies to obtain CSF.

It has been suggested that differing findings in the field may relate to this variation in methodologies (Masdeu *et al.*, 2012, Rhoads *et al.*, 2011). All are based on visualisation of staining, where serum antibody is reacting with a transfected cell expressing the antigen in a natural conformation, but many use fixed permeabilised cells, which may reduce sensitivity and potentially may reduce specificity.

We noted the absence of antibody reactivity in all the plasma samples in cases. This may reflect a true absence of positive samples or could result from an unidentified technical problem. Antibodies can be tested adequately in plasmapheresis samples but the plasma samples were collected in EDTA tubes; it is theoretically possible that this may have affected the live cell assays. We decided that it was important to report this group as, were we to exclude them, while the proportion of positive cases and controls would increase further, there would still be no difference between groups.

During the course of the study patients with organic presentations were excluded which may be one reason for the low proportion of positive results in comparison with Lennox *et al.* 2017 (Lennox *et al.*, 2017). Further, as the proportion of cases and of controls with antibody positivity was small, it remains a possibility that the negative findings may be due to insufficient statistical power. We do not have longitudinal data in relation to outcomes for these patients and none of the patients received immunotherapy concurrent to the identification of antibody positivity. We did not set out to describe clinical impact and course of illness in those with antibody positivity.

Conclusions

We have identified a similar low prevalence of NMDAR and VGKC-complex antibodies in patients with first episode psychosis as in controls. Thus screening of patients with FEP for these antibodies in isolation will generate results that may be difficult to interpret in practice. Further work is needed to understand what other conditions, either clinical or biological, need to exist to render such antibodies clinically relevant in psychosis.

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FG has received honoraria for advisory work and lectures from Roche, BMS, Lundbeck, Otsuka and Sunovion, is a collaborator on a NHS Innovations project co-funded by Janssen and has a family member with professional links to Lilly and GSK, including stock

RSF is now an employee of Otsuka Pharmaceuticals.

EP is an employee of Mundipharma Research Limited, Cambridge, UK

RMM has received payment for lectures including service on speakers' bureaus for BMS, Janssen, AZ.

AV and the University of Oxford hold patents and receive royalties for antibody tests including LGI1 and CASPR2.

The other authors, JL, KB, RM, PGS, EC,LJ, BL, MF and TN have no conflict of interest to declare.

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