



## Antibodies to retroviruses in recent onset psychosis and multi-episode schizophrenia

Faith Dickerson <sup>a,\*</sup>, Erik Lillehoj <sup>b</sup>, Cassie Stallings <sup>a</sup>, Melanie Wiley <sup>b</sup>, Andrea Origoni <sup>a</sup>, Crystal Vaughan <sup>a</sup>, Sunil Khushalani <sup>a</sup>, Sarven Sabuncuyan <sup>c</sup>, Robert Yolken <sup>c</sup>

<sup>a</sup> The Stanley Research Program at Sheppard Pratt, Baltimore, MD, USA

<sup>b</sup> University of Maryland School of Medicine, Baltimore, MD, USA

<sup>c</sup> The Stanley Neurovirology Laboratory, Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD, USA

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### ABSTRACT

**Background:** Immunological abnormalities involving the upregulation of endogenous retroviruses have been associated with schizophrenia in small studies.

**Methods:** Blood samples from 666 individuals (163 with recent onset psychosis, 268 with multi-episode schizophrenia, and 235 controls) were assayed for IgG antibodies to murine leukemia virus (MuLV), Mason-Pfizer monkey virus (MPMV), and feline immunodeficiency virus (FIV) by enzyme immunoassay utilizing whole virus and viral components. Antibody levels in the psychiatric groups were compared to controls by multivariate linear regression. Odds ratios associated with increased antibody levels were calculated based on values  $\geq 75$ th percentile of the controls. Samples were also tested for antibodies to viral proteins by Western blotting and for DNA from infectious retroviruses by real time PCR. Homology between the target virus and the prototype human genome was determined using sequence analysis methods.

**Results:** Compared with controls, individuals with recent onset of psychosis had increased levels of antibodies to MPMV and MuLV (both  $p < .001$  adjusted for covariates), and increased antibody levels for defined portions of the MPMV and MuLV gag, pol and env proteins. The specificity of these antibodies was confirmed by Western blotting. Individuals with multi-episode schizophrenia did not show elevated antibody levels to any of the retroviruses measured. Infectious retroviruses were not detected in the blood of any participants. Homology analyses indicated that there are multiple regions of the human genome homologous with MPMV and MuLV proteins, the highest being with the MuLV gag protein.

**Conclusions:** Antibodies to retroviral proteins are elevated in individuals with recent onset psychosis but not in individuals with multi-episode schizophrenia. The immunopathological consequences of this antibody response should be the subject of additional studies.

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### 1. Introduction

Schizophrenia is a pervasive neurodevelopmental disorder associated with excess morbidity and mortality throughout the world. Family and adoption studies have provided evidence for a genetic component for schizophrenia and genetic linkage studies have identified a number of chromosomal regions which are associated with the disorder. However, few specific genes have been found which confer a major risk for schizophrenia in different populations. Some of the genes which have been found are components of the immune response (Tiwari et al., 2010). In other studies, immunological and infectious factors have been identified which may contribute to the pathophysiology of schizophrenia in some individuals (Torrey and Yolken, 2001; Knight et al., 2007; Patterson, 2009).

Endogenous retroviruses are elements of the human genome which arose from the retro-transposition of exogenous retroviruses following the infection of germ line cells in primate progenitors of modern humans (Bannert and Kurth, 2004). Endogenous retroviruses are present at multiple sites within the human genome. Furthermore, endogenous retroviruses are transcriptionally active during fetal development, a time critical for the formation of neural circuitry which can be disrupted in some individuals with schizophrenia. Previous studies have documented the aberrant expression of endogenous retroviruses in small numbers of individuals with persistent schizophrenia from blood samples (Perron et al., 2008), cerebrospinal fluid (Karlsson et al., 2001), and post-mortem brain tissue (Yolken et al., 2000; Karlsson et al., 2001) and also in small numbers of individuals with recent onset schizophrenia from blood samples (Yao et al., 2008) and cerebrospinal fluid (Karlsson et al., 2001).

The measurement of antibodies to endogenous retroviruses would provide a method for the measurement of activation of retroviral proteins in a large number of individuals. While the measurement of

\* Corresponding author at: Sheppard Pratt, 6501 North Charles St., Baltimore, MD 21204, USA. Tel.: +1 410 938 4359; fax: +1 410 938 4364.

E-mail address: [fdickerson@sheppardpratt.org](mailto:fdickerson@sheppardpratt.org) (F. Dickerson).

antibodies to endogenous proteins is hampered by the lack of available reagents, there are a number of cultivable animal retroviruses which share homology to endogenous retroviruses and can thus be used for the measurement of antibodies. We measured antibody levels to antigens encoded by the *gag*, the *pol* and the *env* genes. These genes, which play essential roles in infection, replication and genome integration, are encoded by all retroviruses. The *gag* (which is an abbreviation for “group specific antigen”) gene encodes the structural protein of the retrovirus. The *pol* gene codes for the reverse transcriptase enzyme required for converting RNA back into DNA. In most retroviruses, this gene also encodes a viral protease required for the processing of viral proteins and efficient cellular infection as well as other proteins such as ribonuclease H and viral integrases. The *env* gene encodes the surface glycoprotein and the transmembrane domains of the virion. These glycoproteins interact with host receptors and facilitate infection (Hughes and Varmus, 1997).

In this study we examine the levels of antibodies to three retroviruses, murine leukemia virus (MuLV), Mason-Pfizer monkey virus (MPMV), and feline immunodeficiency virus (FIV) and their components in a large group of individuals with recent onset of psychosis and with multi-episode schizophrenia. We compare the antibody levels in both psychiatric groups to the antibody levels in controls who do not have any history of psychiatric disorder.

## 2. Methods

The study population consisted of 666 individuals: 163 with a recent onset of psychosis; 268 with multi-episode schizophrenia, not of recent onset (referred to from here on as the “schizophrenia” group); and 235 controls without a history of psychiatric disorder. The details of the recruitment and evaluation of individuals in these populations have been previously described (Dickerson et al., 2003, 2008; Severance et al., 2011). The recent onset of psychosis sample met the following criteria: onset of psychotic symptoms for the first time within the past 24 months defined as the presence of a positive psychotic symptom of at least moderate severity that lasted through the day for several days or occurred several times a week and could not have been limited to a few brief moments; age between 13 and 45 inclusive; absence of substance-induced psychosis or of psychotic symptoms which occurred only in the context of intoxication or withdrawal. All of the recent onset patients were receiving antipsychotic medication at the time of study participation. The individuals with schizophrenia met the following criteria: age between 18 and 65 inclusive, diagnosis of schizophrenia or schizoaffective disorder meeting criteria with the Structured Clinical Interview for DSM-IV Axis I Disorders—Patient Edition (First et al., 1998a); onset of psychotic symptoms more than 24 months earlier; currently receiving antipsychotic medication treatment. The non-psychiatric control sample met the criteria: age between 20 and 60 inclusive, absence of a current or past psychiatric disorder as confirmed by screening with the Structured Clinical Interview for DSM-IV Axis I Disorders—Non-patient Edition (First et al., 1998b). All participants from the patient groups and the control group met the following additional criteria: absence of any history of intravenous substance abuse; absence of primary diagnosis of substance abuse or dependence; absence of mental retardation; absence of serious medical disorder such as stroke, current cancer, or HIV infection. The participants with recent onset psychosis were recruited from inpatient and day hospital programs at a large psychiatric health system. The participants with schizophrenia were recruited from psychiatric programs affiliated with the same psychiatric health system and at other outpatient treatment sites in the region. The control individuals without a history of psychiatric disorder were recruited from posted announcements at local health care facilities and universities in the same geographic area as the sites where the individuals with recent onset psychosis and those with schizophrenia were drawn. The controls

were not selected on the basis of the presence or absence of non-psychiatric disorders.

Participants were asked about their educational level and other demographic variables. Participants in the patient psychiatric groups were also interviewed and rated on the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) to assess current psychiatric symptom. Medication data were recorded from their clinical charts.

The studies were approved by the Institutional Review Boards of the Sheppard Pratt Health System and the Johns Hopkins Medical Institutions following established guidelines. All participants provided written informed consent after the study procedures were explained.

A blood sample was obtained at the study visit for all participants. The samples were assayed for IgG antibodies to murine leukemia virus, Mason-Pfizer monkey virus, and feline immunodeficiency virus by enzyme immunoassay utilizing whole virus and viral components as further described below.

Purification of virus proteins: MuLV, MPMV, and FIV whole viruses (American Type Culture Collection, Manassas, VA) was resuspended in SDS-PAGE sample buffer and proteins were resolved on 15% preparative polyacrylamide gels. Proteins in gel slices were recovered by electroelution at 100 V for 2 h in 0.05 M Tris-HCl, pH 7.0, 10 mM glycine. Individual viral proteins were identified by analytical SDS-PAGE and ELISA using goat antisera specific for envelope (*env*) and *gag* proteins (Quality Biotech, Camden, NJ). Protein concentrations are measured by the method of Bradford (1976).

Enzyme immunoassay (ELISA): MuLV, MPMV, and FIV whole viruses and purified proteins were resuspended at 10 µg/ml in 0.05 M sodium carbonate, pH 9.6 and 100 µl/well incubated overnight at 4 °C in flat-bottom 96-well microtiter plates (Dynatech, Chantilly, VA). The wells were blocked with 100 µl of 1% BSA (Sigma, St. Louis, MO) and 1% sucrose for 1 h at room temperature and washed 3 times with 1.5 mM imidazole, pH 7.4, 37.8 mM NaCl, 0.025% Tween 20. Patient and control serum samples were diluted 1/100 (v/v) in 0.05 M PBS, pH 7.2 containing 0.3% Triton X-100, 0.2% Tween 80, and 1.0% BSA (MB buffer) and 100 µl/well incubated for 1 h at room temperature. Wells were washed 3 times and bound antibody reacted with 100 µl of peroxidase-conjugated goat anti-human IgG antibody (KPL, Gaithersburg, MD) diluted 1/3000 in MB buffer for 1 h at room temperature and with 100 µl of ABTS substrate (KPL) added for 30 min. OD at 405 nm was measured with an automated microplate reader. The samples were tested under code with the persons performing the tests unaware of the diagnosis of the sample donor.

Western blotting: MuLV and MPMV whole virus proteins were resolved on 10% SDS-acrylamide gels and transferred to polyvinylidene-diamine (PVDF) membrane in 25 mM Tris-HCl, pH 8.3, 0.192 M glycine and 20% methanol as described (Towbin et al., 1979). The membranes were blocked with 5% non-fat dry milk in PBS and cut into 3 mm strips. Patient and control serum samples were diluted 1/100 (v/v) in 5% milk in MB buffer and 1.0 ml/strip was incubated overnight at 4 °C. The strips were washed 3 times, bound antibody was reacted with 100 µl of peroxidase-conjugated goat anti-human IgG antibody (KPL) diluted 1/10,000 in MB buffer for 1 h at room temperature and detected with enhanced chemiluminescence (ECL) substrate (Amersham, Piscataway, NJ).

Polymerase chain reaction (PCR): DNA was extracted from whole blood and tested for the presence of proviral nucleic acids homologous to MuLV and MPMV using real time polymerase chain reactions and gel following established procedures (Lerche et al., 2001). The primers and probes used for the detection of MPMV have been previously described (Lerche et al., 2001). Two sets of primers and probes were used for the detection of MuLV. Set 1: forward primer 5'-AAATCTTCTAACCGCTCTAACTTTCG-3'; reverse primer: 5'-AAACCATGTGtCCATGTCATT-3'; labeled probe: FAM5'-ATCTGGCAGTtGCCCGG-3'TAMRA. Set 2: forward primer: 5'-TCTTTGTTCGACGAGAAGCAG-3'; reverse primer: 5'-CTGCCACTGGTCTAGCTTT-3'; labeled probe. FAM5'-CGCCAAAGG-CGTCTAACGC-3'TAMRA. These primers were selected not to amplify

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