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Viral metagenomics in drug-naïve, first-onset schizophrenia patients with prominent negative symptoms

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ABSTRACT

Although several studies suggest a virus or (endogenous) retrovirus involvement at the time of onset of schizophrenia, the unequivocal identification of one or more infectious agents, by means of an undirected catch-all technique, has never been conducted. In this study VIDISCA, a virus discovery method, was used in combination with Roche-454 high-throughput sequencing as a tool to determine the possible presence of viruses (known or unknown) in blood of first-onset drugs-naïve schizophrenic patients with prominent negative symptoms. Two viruses (the *Anellovirus* Torque Teno virus and GB virus C) were detected. Both viruses are commonly found in healthy individuals and no clear link with disease was ever established. Viruses from the family *Anelloviridae* were also identified in the control population (4.8%). Besides, one patient sample was positive for human endogenous retroviruses type K (HML-2) RNA but no specific predominant strain was detected, instead 119 different variants were found. In conclusion, these findings indicate no evidence for viral or endogenous retroviral involvement in sera at the time of onset of schizophrenia.

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1. Introduction

Schizophrenia is a severe and disabling mental disorder of largely unknown aetiology. Gene–environment interactions may be involved in the development of vulnerability and prominent negative symptoms may be regarded as a characteristic of severe type of the disorder, possibly associated with neurodegeneration (Tsuang, 2000; Huang et al., 2011). Connections between the onset of schizophrenia and viral infections are reported, indicating that viral infections may play a role in the etiopathogenesis of schizophrenia (Arias et al., 2012).

Within the main framework of the 'viral hypothesis' of schizophrenia, different etiopathogenetic hypotheses may be distinguished. First, it can be hypothesized that a viral infection, or the immunological response to it, acquired in the prenatal/perinatal period or post-partum may (subtly) damage the maturation of the brain, resulting in overt symptoms starting in adolescence (Canuti et al., 2015). An evidence for this hypothesis is the 5–8% increased risk of developing schizophrenia among those born in the winter–spring months, when infectious diseases are more prevalent (O'Callaghan et al., 1991). Moreover, serological studies have shown an elevation in risk of schizophrenia following exposure to several agents during gestation (Brown et al., 2004a, 2005; Buka et al., 2008; Brown and Derkits, 2010). Besides, some studies reported an association between higher level of

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inflammatory cytokines during pregnancy and schizophrenia associated problems in the offspring (Buka et al., 2001; Brown et al., 2004b).

A second theory hypothesize that a persistent viral infection, continuously affecting brain maturation, leads to overt symptoms in the adolescent period and, thirdly, it is possible that a dormant virus is (re-)activated in adolescence, giving rise to overt symptoms. In fact associations between schizophrenia and pathogenic agents have been reported, like in the cases of human herpes virus, cytomegalovirus, measles virus and *Toxoplasma gondii* (Dickerson et al., 2003, 2010; Torrey et al., 2006; Blomström et al., 2012; Khademvatan et al., 2014). Finally, a recently conducted meta-analysis-including studies performed on serum/blood, brain tissue and CSF- showed statistically significant association between schizophrenia and infection by human herpes virus 2, Borna Disease Virus and Human Endogeneous Retrovirus (HERV) type W (Arias et al., 2012).

A possible involvement of HERVs in schizophrenia is a new area in the field of schizophrenia research (Leboyer et al., 2013). HERVs are ancient retroviruses of which the integrated provirus has become a stable element of the host genome (about 8% of the human genome, (Subramanian et al., 2011)). Although the majority of them have become mutated and truncated, a few have retained the capacity to encode viral proteins, still contain intact promoters or are able to form virus-like particles (Boller et al., 2008). Moreover, novel HERV loci can be generated by recombination/(retro) transposition during gestation, creating modifications in the embryonic DNA (Subramanian et al., 2011), and some HERVs are mobile genetic elements which may be regulated/activated by environmental factors, such as microbial agents, and can induce genetic rearrangements (Huang et al., 2011; Feschotte and Gilbert, 2012). An association between endogenous retrovirus activation and schizophrenia has been supported by several studies (Lillehoj et al., 2000; Yolken et al., 2000; Karlsson et al., 2001, 2004; Perron et al., 2008; Leboyer et al., 2013). The HERV type K family, the retrovirus family most recently integrated in the human genome, encompasses the HML-2 group. The expression levels of HML-2 viruses may be up-regulated in schizophrenic patients (Subramanian et al., 2011) and HERV-K10 has been found to be significantly over-expressed in brain samples of schizophrenia patients when compared to healthy controls (Frank et al., 2005).

Recent advances in molecular-biological techniques enable the simultaneous screening for all viruses, in a non-hypothesis driven, high-throughput manner. One such technique is VIDISCA-454, a sequence independent and high-throughput sequencing based virus discovery technique that provides a fast and effective tool for the amplification and the identification of both DNA and RNA viruses (Canuti et al., 2011, 2014a, 2014b; De Vries et al., 2011, 2012; Jazaeri Farsani et al., 2013, 2015; Tan et al., 2013; Oude Munnink et al., 2014). In the present study VIDISCA-454 was used as an unbiased approach to study the involvement of any virus (known, unknown or actively replicating/expressing endogenous retroviruses) in sera of first-onset schizophrenic patients with prominent negative symptoms. Since previous studies have reported that the use of some antipsychotic drugs may up-regulate the transcription activity of some endogenous retroviruses (Diem et al., 2012), we selected only antipsychotic drugs-naïve patients.

2. Methods

2.1. Patient selection

Patients were recruited at the department of psychiatry at the Erasmus University Medical Center, Rotterdam (EMC). The clinical and demographic state of the patients was evaluated at baseline. Severity of symptoms was assessed by the Positive And Negative Syndrome Scale by trained experienced investigators who were blind to outcome results. Eligible for inclusion were all patients diagnosed with schizophrenia according to DSM-IV criteria after a Comprehensive Assessment of Symptoms and History interview (CASH) (Andreasen et al., 1992). For patients who had symptoms for less than 6 months the final diagnosis was made after 6 months to comply with the DSM-IV criterion. Additional criteria were recent onset (defined as duration of illness <5 yr), a score on the negative symptoms subscale of the Positive And Negative Syndrome Scale (PANSS) of 21 or higher to ensure prominent negative symptoms and age >15 and <36 years. All patients were antipsychotic naive and exclusion criteria were the presence of any somatic or neurological disorder. The study was approved by the standing ethics committee, and all the subjects gave written informed consent in accordance with the committee's guidelines.

Twenty-three drug naïve, first onset schizophrenic patients were selected for the study (males to females ratio was 21/2, mean age was 24.28, SD 5.12). The mean PANSS was 80.3 (SD 17.6) and for negative score was 19.8 (SD 6.9).

2.2. Controls

As a control for virus discovery and molecular studies anonymized serum samples from healthy Dutch blood donors were used ($N=84$ for virus discovery, $N=29$ for the molecular studies). All donors donated blood voluntarily and no monetary compensation was involved.

2.3. Virus discovery: VIDISCA-454

Serum samples collected from recent onset schizophrenia patients and controls were subjected to virus discovery techniques in order to identify any actively replicating virus. Pools were prepared from patients serum samples ($N=23$: 5 pools of 4 samples and one pool of 3 samples) and from controls ($N=84$: 42 pools of 2 samples) and VIDISCA was performed as previously described (De Vries et al., 2011). Briefly, 110 μl of each pool was spun down to remove intact cells and 100 μl of the supernatant was subjected to DNAse treatment to eliminate background cellular DNA with 20 U TURBO™ DNase (Ambion). Nucleic acids were extracted from the pre-treated samples as described by Boom (Boom et al., 1990). In order to subsequently detect RNA viruses a reverse transcription with 200 U of Superscript II (Invitrogen) and non-ribosomal hexamers (Endoh et al., 2005) was performed followed by a second strand synthesis with 5 U of Klenow frament (3′-5′ exo-) (New England Biolabs) and 7.5 U of RNase H (New England Biolabs). Samples were purified by a phenol chloroform extraction and ethanol precipitation. Double stranded DNA was digested with 10 U of *MseI* (New England Biolabs) restriction enzyme and A- and B-adaptors were specifically ligated to the obtained fragments. A-adaptors contained the Roche-454 primer A binding site (CGTATCGCCTCCCTCGCGCCA), the Roche-454 key sequence (TCAG), a 10 nt specific MID (Multiplex Identifier, different MIDs were assigned to each pool) and the specific *MseI* ligation overhang; every B adaptor contained the Roche-454 primer B binding site (CTATGCGCCTTGCCAGCCCGC) and the specific *MseI* ligation overhang. A PCR was performed by using Roche-454A and B primers and size selections were purified from 1% agarose gel by the use of NucleoSpin® Extract II (Macherey-Nagel) purification kit. Purified DNA was quantified with the Quant-iT™ dsDNA Assay Kit on a Qubit fluorometer (Invitrogen). Emulsion PCR and sequencing PCR were performed according to the suppliers protocol (LIB-A SV emPCR kit, GS FLX Titanium XLR 70 Sequencing kit, Roche). After bead counts, samples with a bead recovery between 5% and 20% were used for 454 sequencing (Roche) on a 4-region GS FLX

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