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Human endogenous retroviral pol RNA and protein detected and identified in the blood of individuals with schizophrenia

Wen-Jie Huang [a,1], Zhong-Chun Liu [b,1], Wei Wei [a], Gao-Hua Wang [b], Jian-Guo Wu [a,*], Fan Zhu [a,*]

[a] *College of Life Sciences, Wuhan University, Wuhan 430072, People's Republic of China*
[b] *Ren-Ming Hospital, Wuhan University, Wuhan 430060, People's Republic of China*

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Abstract

Retrovirus has been speculated as one of the potential infectious agents involved in the development of schizophrenia. Here we used nested RT-PCR to detect the RNA of HERV *pol* gene in blood from schizophrenic patients and normal human. We found retroviral *pol* genes expressed in blood from 20 of 58 (34.5%) individuals with recent-onset schizophrenia, but not from 38 normal persons ($p<0.01$). Sequence analysis revealed that the expressed gene was homologous to those of the human endogenous retroviral (HERV) family. The ERV9 family was the closest, with 90% homology in the gene sequence. In addition, Western blots showed that antibody against ERV9 pol protein in serum from the HERV+ schizophrenia patients, but not from control ($p<0.01$). Our data suggested that the transcriptional activation of certain retroviral elements might be associated with the development of schizophrenia in some patients. Further characterization of retroviral elements in subjects with schizophrenia may aid in better diagnosis and treatment of this disorder.

Keywords: Schizophrenia; HERV; pol

Schizophrenia is a psychiatric disorder characterized by disturbances in multiple domains of brain function, including cognitive, emotional, and perceptual processes (Lewis, 2001; Karlsson et al., 2001). The exact etiology of this disease is not fully understood (Karlsson, 2003). Some retroviruses (such as HTLV and HIV) have been shown to impair the immune system and provoke central nervous system inflammation and/or degeneration (Brodsky and Foley, 1993). Retroviruses have also been suggested as one of the infectious agents contributing to schizophrenia (Yolken, 2004). In addition, it was also known that the tissue-specific expression of HERV had been associated with a number of chronic illnesses, such as multiple sclerosis, diabetes, autoim-

* Corresponding authors. Tel.: +86 27 68754600; fax: +86 27 68752560.
E-mail address: zhufan@hotmail.com (F. Zhu).
[1] These authors contributed equally to the work.

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mune arthritis etc. (Perron et al., 1997; Nakagawa et al., 1997; Conrad et al., 1997). In order to explore the potential connection between schizophrenia and HERV, we tested for retroviral components in blood samples taken from schizophrenia patients. We found both mRNA and protein of *pol* gene of retrovirus belonging to the HERV family present in subjects with recent-onset schizophrenia but not in normal individuals in China.

1. Materials and methods

1.1. Patients and normal individual blood samples

All patients were admitted in RenMin Hospital, Wuhan University. Blood samples were taken from 58 patients exhibiting symptoms consistent with recent-onset schizophrenia as defined by the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Ed. They had not been admitted to the hospital previously for schizophrenia and showed no manifestations of acute infectious, inflammatory, or neurological diseases before their admission. The median age of these individuals was 21 years old (ranging from 15 to 32). They came from different areas in China (20 from Hubei, 5 from Beijing, 10 from Hunan, 1 from Guangdong, 6 from Sichuan, 12 from Shanghai, and 4 from Hainan). Blood samples from 38 normal individuals randomly selected by a physical examination displaying no evidence of neurological or psychiatric diseases were also obtained from RenMing Hospital, Wuhan University. The median age of these individuals was 25 years (ranging from 18 to 46). They also came from different areas in China (11 from Hubei, 6 from Beijing, 5 from Shandong, 6 from Shanghai, 4 from Shanxi, 4 from Hunan and 2 from Jiangsu). All blood samples were separated into two fractions: serum and leukocytes. Samples were all stored at −80 °C for analysis.

1.2. RNA isolation/cDNA synthesis

As previously described, total RNA was isolated with Trizol LS Reagent (GIBCO BRL) and treated with DNase I to remove genomic DNA (Zhu et al., 2004). cDNA was synthesized as described (Zhu et al., 2004, 2002).

1.3. Nested-PCR amplification

For the first round of amplification, PCR was performed with a pair of primers, P1 (5′-TGGTT/C/TA/TCA/GGTC/TC/TTA/GGACC/TT-3′) and P2 (5′-A/GTCATCCAC/TA/GTAC/TTGA/TAGGA-3′). One microliter of the product was then used for the second round PCR with another pair of primers, P1 and P3 (5′-CCAAAC/TAA/GATGA/GGGA/GCTATC-3′). The following thermal cycling conditions were used: heat activation of the polymerase for 5 min at 94 °C; followed by 35 cycles of 94 °C for 45 s, 45 °C for 45 s, and 72 °C for 45 s; with a final extension at 72 °C for 10 min. The final product from the second round PCR was analyzed by electrophoresis on 2% agarose gel.

1.4. Cloning, sequencing and analysis

Final PCR products were ligated directly into pGEM-T vector (Promega, USA). The recombinant plasmids were sequenced on Perkin-Elmer 373A automated DNA sequencer (Perkin Elmer, USA). Sequences were analyzed for homology using BlastN (available at http://www.ncbi.nlm.nih.gov/BLAST). Phylogenetic trees were computed with the neighbor-joining method (Saitou and Nei, 1987). DNA sequences of the retroviruses were retrieved from the GenBank database through BLAST network server (Altschul et al., 1997).

1.5. Expression and purification of ERV9 pol fusion proteins

ERV9 *pol* sequence was amplified from cDNA by nested-PCR using P1 and P4 (5′TGCCTCGAG-CAGTTGTCTGATAGCCATAA-3′) as primers' for the first round and P4 and P5 (5′-TTGGAATTCATG-GATCCCTGGATACAGCGAG-3′) for the second round. The PCR product was cloned into expression vector pET28a. After verification by restriction enzyme digestion and DNA sequencing, the recombinant plasmid was transformed into competent *E. coli* strain BL21 (DE3) cells by standard procedure (Sambrook et al., 1989). Fusion proteins were expressed and purified as described (Zhu et al., 2005) except that Ni-NTA His Bind Resin was used in purification.