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Identification and Phylogenetic Analysis of Long Terminal Repeat Elements of the Human Endogenous Retrovirus K Family (HERV-K) from a Human Brain cDNA Library

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Long terminal repeats (LTRs) of the human endogenous retrovirus K family (HERV-K) have been found to be coexpressed with sequences of genes closely located nearby. We examined transcribed HERV-K LTR elements in human brain tissue. Using cDNA synthesized from mRNA of the human brain, we performed PCR amplification and identified ten HERV-K LTR elements. These LTR elements showed a high degree of sequence similarity (92.4-99.7%) with the human-specific LTR elements. A phylogenetic tree obtained by the neighbor-joining method revealed that HERV-K LTR elements could be divided into two groups through evolutionary divergence. Some HERV-K LTR elements (HKL-B7, HKL-B8, HKL-B10) belonging to the group II from human brain cDNA were closely related to the human-specific HERV-K LTR elements. Our data suggest that HERV-K LTR element are active in the human brain; they could conceivably play a pathogenic role in human diseases such as psychosis.

The HERV-K family of human endogenous retroviral sequences was originally cloned from Syrian hamster intra-cisternal A type particles (Ono et al., 1986), has homology to mouse mammary tumour virus, and includes sequences that are expressed in normal placenta and leukemic cells (Simon et al., 1994). HERV-K elements probably entered the primate genome after the split of New World monkeys in the Oligocene era, 33-40 million years ago (Steinhuber et al., 1995). It is estimated that there are now approximately 25,000 copies of HERV-K LTRs in the human genome, and they have randomly transposed across the chromosomes in the course of human evolution (Leib-Mösch et al., 1993). The possibility that some of this increase has occurred recently was suggested by the presence of a functional integrase in HERV-K sequences (Kitamura et al., 1996). The ratio of synonymous to non-synonymous substitutions suggests that evolutionary selection has recently been or is still operating (Zsiros et al., 1998). Medstrand and Mager (1998) have demonstrated that some clusters of HERV-K LTRs show low (average 1.5%) divergence; in comparisons across primate species, members of their cluster 9 are specific to the Homo sapiens. HERV-K LTRs bind host cell nuclear proteins and have the potential to activate neighboring genes (Akopov et al., 1998). A possible role of HERV-K elements in diseases has been considered in relation to insulin dependent diabetes mellitus (Conrad et al., 1997), seminoma (Sauter et al., 1995) and HERV-K-T47D mammary carcinoma (Seifarth et al., 1998). Here we identified ten HERV-K LTR elements transcribed in human brain tissue and analyzed them phylogenetically with other HERV-K LTR family. This study had its origin in the hypothesis that an endogenous retroviral element has relevance to psychosis such as schizophrenia (Crow, 1984; Yee et al., 1998; Kim et al., 1999).

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Materials and Methods

PCR amplification for HERV-K LTR elements

The cDNA synthesized from mRNA of human brain (Clontech) was used as a template for PCR amplification. New HERV-K LTR elements were amplified by the specific primer pair HS45 (5′-GTATTGTCCAAGGTTTCTCCC-3′, bases 8541-8561) and DS14 (5′-GTGCTGTGCTTTTGGATATGC-3′, bases 8859-8879) from the human endogenous retroviral element, HERV-K10 (GenBank, accession no. M14123).

Molecular cloning of PCR products

PCR products were separated on 2% agarose gel, purified with the QIAEX II gel extraction kit (Qiagen) and cloned into the T-khs307 vector (Kim et al., 1998). The cloned DNA was isolated by the alkali lysis method using the High Pure plasmid isolation kit (Boehringer Mannheim). Individual plasmid DNAs were screened for inserts by PCR using the original primers designed for the locus.

DNA sequencing and data analysis

Positive samples were subjected to sequence analyses on both strands with T7 and M13 reverse primers using an automated DNA sequencer (Model 373A) and the DyeDeoxy terminator kit (Applied Biosystem). Nucleotide sequence analysis was performed using the GAP and PILEUP programs of the GCG software (Genetics Computer Group, University of Wisconsin). The neighbor-joining phylogenetic analysis was performed with the MEGA program (Kumar et al., 1993). Nucleotide sequences of HERV-K LTR elements were retrieved from the GenBank database with the aid of BLAST network server (Altschul et al., 1997).

Results and Discussion

Using cDNA from human brain tissue as a template, PCR with HERV-K LTR specific primers resulted in the amplification of the expected 341 bp fragments. We cloned HERV-K LTR elements from the PCR products and determined their nucleotide sequences. Ten HERV-K LTR elements were identified newly from the human brain cDNA library. They have a high degree of sequence similarity (92.4-99.7%) with the human-specific LTR elements (AC002350, AC002400, AC002508, AL034407, L47334, U47924, Z80898) (Table 1). In human genome, several thousand copies of HERV-K LTR elements are randomly distributed. We have already identified some of the LTR elements using human monochromosome and genomic DNAs (Kim and Crow 1999; Kim et al., 2000). Medstrand and Mager (1998) have also identified human-specific HERV-K LTR elements (AC002350, AC002400, AC002508, etc.) from the GenBank database. All those LTR elements were used to construct a phylogenetic tree with the ten new HERV-K LTR elements that were transcribed in human brain tissue. As shown in Fig. 1, the HERV-K LTR elements were mainly divided into two groups through evolutionary divergence. The human-specific HERV-K LTR element (AL034407), which proliferated in human Xq26 recently (Kim et al., 2000), belonged to group II. Similarly, other human-specific HERV-K LTR elements (AC002350, AC002400, AC002508, L47334, U47924, Z80898) (Medstand and Mager, 1998) also belonged to group II. Some HERV-K LTR elements (HKL-B7, HKL-B8, HKL-B10), identified from the human brain cDNA in this study, were closely related to the human-specific HERV-K LTR elements within the group II. The data allow us to speculate that new HERV-K LTR elements are active in human brain tissue and may represent a source of genetic variation connected to human disease such as psychosis. In our previous study, we investigated the retroviral/retroposon hypothesis of schizophrenia by generating sequences with PCR primers based on a retroviral sequence recovered by Yee et al. (1998). The retroviral sequence derived from a cDNA library from postmorte brain tissue of an individual with psychosis in a genomic region (Xq21.3). The Xq21.3 region has been tentatively linked to schizophrenia and schizoaffective disorder by Laval et al. (1998). We identified two retroposons, HS307 and HS408, on human Xq21. 3 region. They had a high degree of sequence similarity with the schizophrenic brain cDNA sequences. Moreover, the HS307 retroposon was very closely related to SINE-R.C2 that was found as a human-specific retroposon (Zhu et al., 1994). The SINE-R.C2 retroposon family was shown to be derived from HERV-K LTR elements by phylogenetic analysis (Kim et al., 1999). Therefore, we were interested in exploring the HERV-K LTR elements from human

Table 1. Similarity (%) of nucleotide sequences of HERV-K LTRs

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. HERV-K10LTR	–																	
2. HKL-B1	94.9	–																
3. HKL-B2	95.3	99.7	–															
4. HKL-B6	97.0	93.9	94.3	–														
5. HKL-B7	98.0	94.3	94.6	95.6	–													
6. HKL-B8	98.0	95.6	96.0	96.3	97.3	–												
7. HKL-B9	94.1	92.4	92.7	94.4	92.7	93.4	–											
8. HKL-B10	99.0	94.6	94.9	96.6	97.6	97.6	93.7	–										
9. HKL-B12	94.0	98.3	98.7	93.0	93.3	94.6	91.4	93.6	–									
10. HKL-B14	94.6	99.0	99.3	93.6	93.9	95.3	92.1	94.3	98.7	–								
11. HKL-B15	94.9	99.3	99.7	93.9	94.3	95.6	92.4	94.6	98.3	99.0	–							
12. AC002350	99.3	94.9	95.3	97.0	98.0	98.0	94.1	99.7	94.0	94.6	94.9	–						
13. AC002400	99.7	95.3	95.6	97.3	98.3	98.3	94.4	99.3	94.3	94.9	95.3	99.7	–					
14. AC002508	99.0	94.6	94.9	96.6	97.6	97.6	93.7	98.7	93.6	94.3	94.6	99.0	99.3	–				
15. AL034407	99.3	94.9	95.3	97.0	98.0	98.0	94.1	99.0	94.6	94.6	94.9	99.3	99.7	99.0	–			
16. L47334	99.0	94.6	94.9	96.6	97.6	97.6	93.7	98.7	94.3	94.3	94.6	99.0	99.3	98.7	99.0	–		
17. U47924	97.6	93.9	94.3	95.3	96.3	97.0	92.4	97.3	93.6	93.6	93.9	97.6	97.3	97.3	97.6	97.3	–	
18. Z80898	97.6	94.6	94.9	95.3	96.6	97.6	93.4	97.3	94.3	94.3	94.6	97.6	97.3	97.3	97.6	97.3	96.6	–

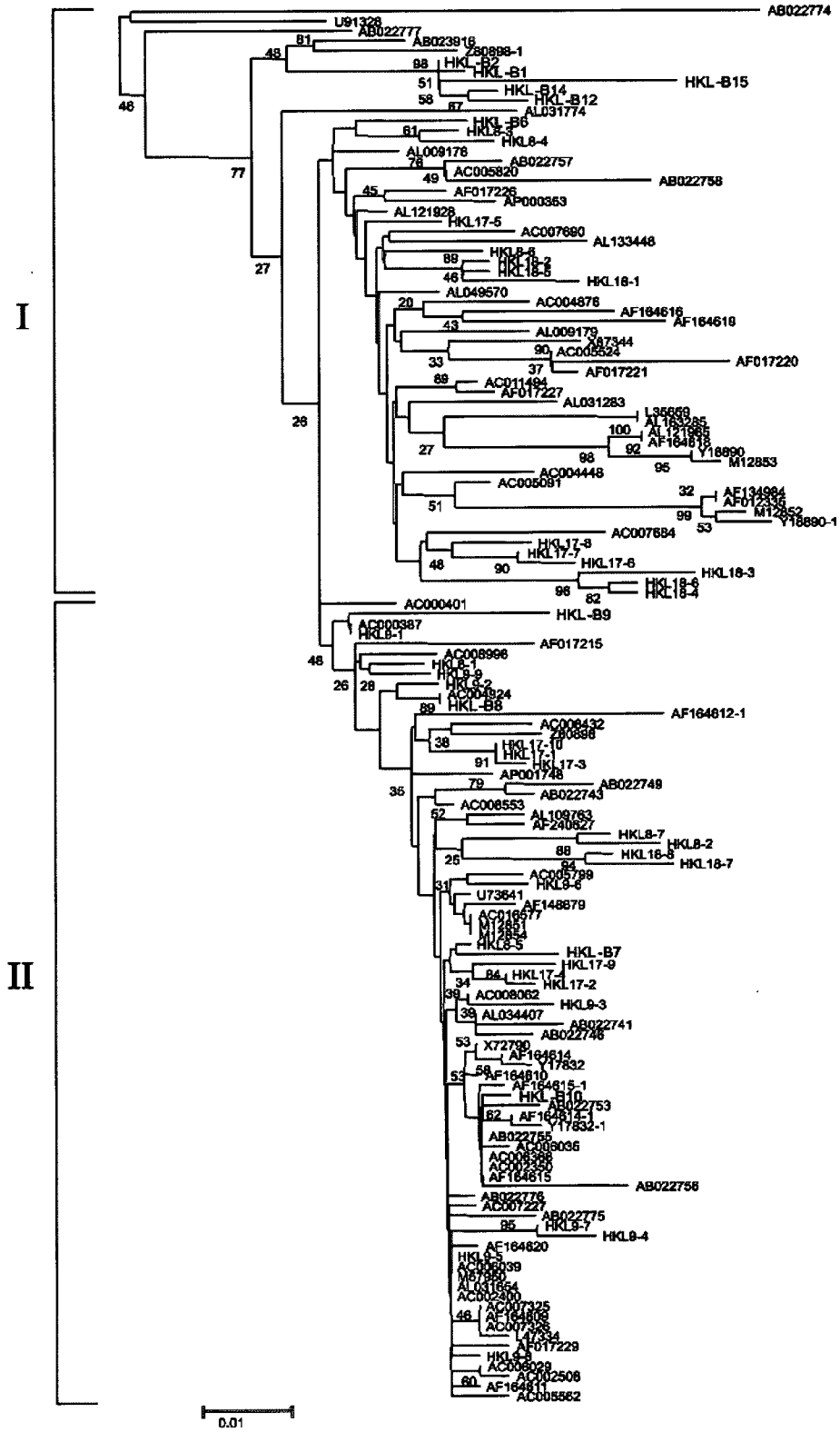

Fig. 1. Phylogenetic tree obtained by neighbor-joining method for the HERV-K LTR family. Branch lengths are proportional to the distances between the taxa. The values at branch-points indicate the percentage support for a particular node after 100 bootstrap replications. The accession numbers of HERV-K LTR elements were obtained from the GenBank database. The cDNA sequence data reported in this paper are in the DDBJ/EMBL/GenBank databases under accession numbers AB052567 (HKL-B1), AB052568 (HKL-B2), AB052569 (HKL-B6), AB052570 (HKL-B7), AB052571 (HKL-B8), AB052572 (HKL-B9), AB052573 (HKL-B10), AB052574 (HKL-B12), AB052575 (HKL-B14), and AB052576 (HKL-B15).

brain. Some new elements in this class were identified in this study as expressed in tissue from the frontal cortex of schizophrenic patient (Yee et al., 1998) although we cannot determine whether the expression of these elements is related to the disease state because of the absence of extensive patient-control studies. Akopov et al. (1998) have noted that such sequences have the capacity to modify the expression of neighboring genes, and suggested that such modifications may have been acquired in the course of human evolution. The HERV-K-T47D-related LTR element has mediated polyadenylation of cellular transcripts (Baust et al., 2000). In the case of another retroelement (the HERV-F LTR element), a similar phenomenon was observed in relation to the Krüppel-related zinc finger gene ZNF195 (Kjellman et al., 1999). Two HERV-K LTR elements have been detected in the human histocompatibility complex locus HLA-DQ (Kambhu et al., 1990). One LTR element (DQ-LTR3) of the HERV-K family at the HLA-DQB1 locus has been associated with rheumatoid arthritis (Seidl et al., 1999). An element described as almost identical to SINE-R.C2 derived from the HERV-K LTR element is the cause of Fukuyama-type muscular dystrophy (Kobayashi et al., 1998). A retroviral sequence was recovered from cDNA from postmortem frontal cortex of an individual suffering from schizophrenia who committed suicide at the age of 34 (Yee et al., 1998). Therefore we suggest that the ten new HERV-K LTR elements identified from cDNA of the human brain in the present study deserve further investigation as potential leads to the neuropsychiatric diseases.

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