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Isolation and Phylogeny of SINE-R Retroposons Derived from Human Endogenous Retrovirus HERV-K Family in Schizophrenia

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SINE-R retroposons have been derived from human endogenous retrovirus HERV-K family and found to be hominoid specific. Both SINE-R retroposons and HERV-K family are potentially capable of affecting the expression of closely located genes. Using the genomic DNA from patients with schizophrenia, we identified 26 SINE-R retroposons and analyzed them with the sequences derived from the hominoid primates. The SINE-R retroposons from schizophrenia showed 89.7-96.6% sequence similarities with the sequence of the schizo-cDNA clone that derived from postmortem tissue from the frontal cortex of an individual suffering from schizophrenia. Phylogenetic analysis using the neighbor-joining method revealed that the new SINE-R retroposons in schizophrenia have proliferated independently during hominid evolution. Such retroposons have great relevance to genomic change connected to human diseases. The data suggest that new SINE-R retroposons identified in schizophrenia deserve further investigation as potential leads on the understanding of neuropsychiatric diseases.

Approximately 1% of the human genome is comprised of endogenous retroviral sequences, and most of them have been integrated into the primate lineage thirty-three or more million years ago (Mager and Freeman, 1995; Steinhuber et al., 1995). The majority of these elements were incorporated as proviral DNA. They rapidly became replication-defective by random accumulation of mutations to be vertically transmitted as junk DNA. It has been suggested that endogenous retroviruses and retroposons have played a role in influencing the functional organization of the human genome (Baltimore, 1985; Sverdlov, 1998).

HERV-K family of the human endogenous retroviral sequences was originally cloned from Syrian hamster intra-cisternal A type particles and found to have had homology to mouse mammary tumour virus (Ono et al., 1986). SINE-R retroposons have been derived from the HERV-K family (Ono et al., 1987; Kim et al., 1999b). SINE-R11, 14, and 19 were isolated by colony blot hybridization using the long terminal repeat and small upstream flanking regions of HERV-K10 as probe (Ono et al., 1987). These elements entered in the genome of hominoid primates after the split of Old World monkeys in the Oligocene period (Kim et al., 1999a, b). SINE-R.C2 element was discovered within the third intron of the gene for C2, the second component of complement, located in class III of the major histocompatibility complex on the short arm of human chromosome 6 (Carroll et al., 1984; Zhu et al., 1992). By Southern blot analysis, SINE-R.C2 has been found to be human specific (Zhu et al., 1994). A retroposon (schizo-cDNA) was detected from a cDNA library from postmortem brain in schizophrenia (Yee et al., 1998). Within the Xq21.3 region, we previously identified two retroposons (HS307 and HS408) by polymerase chain reaction (PCR) approach using YAC clone panel (Kim et al., 1999b). We have subsequently conducted a more extensive investigation of these elements on human chromosomes 7, 13, 17, X, and Y (Kim and Crow, 1999; 2000; Kim et al., 2000a, b) and hominoid primates (Kim et al., 1999a, b, c; Kim and Takenaka, 2001). We were interested in exploring the SINE-R retroposon in schizophrenia in order to understand its implication on various neuropsychiatric diseases. Here we identified 26 SINE-R retroposons from the blood samples from patients with schizophrenia and analysed them phylogenetically with those of the hominoid primates. This study has its origin in the hypothesis that endogenous retroviral elements have relevance to psychosis such as schizophrenia (Crow, 1984; Yee et al., 1998; Kim et al., 1999b).

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Materials and Methods

PCR amplification for SINE-R retroposons

Genomic DNAs from blood samples from patients with schizophrenia were used as a template for PCR amplification. SINE-R retroposons were amplified by the primer pair JS902 (5′-GAGAATGGGCCATGATG-AC-3′, bases 4-22) and JS903 (5′-GATCATTCTTGG-ATGTTTCT-3′, bases 466-485) from schizophrenic brain S11 cDNA clone (GenBank, accession no. AA772777), which has a high level of sequence homology with SINE-R.C2, 11, 14, 19, and HS307/HS408 elements (Ono et al., 1987; Zhu et al., 1994; Kim et al., 1999b). The PCR conditions were those of Kim et al. (1996) with an annealing temperature of 56℃.

Molecular cloning of PCR products

PCR products were separated on a 1.8% 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 (Roche).

Determination of DNA sequence and data analysis

Individual plasmid DNAs were screened for inserts by PCR. Positive samples were subjected to sequence analysis on both strands with T7 and M13 reverse primers using an automatic DNA sequencer (Model 373A) and the DyeDeoxy terminator kit (Applied Biosystem). Sequence analyses were done with the aid of GAP, PILEUP, and PRETTY programs from the GCG software (University of Wisconsin). Neighbor-joining phylogenetic analysis was performed with the MEGA program (Kumar et al., 1993). Pairwise distance of the number of nucleotide substitutions was estimated using the method of Tajima and Nei (1984). Gap sites were ignored in distance estimation by pairwise-deletion option. Statistical significance evaluation of the branching pattern was performed with 1000 replications. Nucleotide sequences of the SINE-R retroposons were retrieved from the GenBank database with the aid of BLAST network server (Altschul et al., 1997).

Nucleotide sequence accession numbers

The nucleotide sequences of new members of the retroposons reported in this paper have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the following accession numbers AB061742-AB061767.

Results and Discussion

SINE-R retroposons were derived from the long terminal repeat (LTR) of the HERV-K family (Ono et al., 1987). Using the bioinformatic tool, we examined that a schizo-cDNA clone derived from the postmortem tissue of the frontal cortex of an individual suffering from schizophrenia (GenBank, accession no. AA772777) was found to have sequences showing a high degree of homology to the SINE-R.C2 (Zhu et al., 1994). Therefore, we designed PCR primers from the schizophrenic brain cDNA. Using the genomic DNAs from patients with schizophrenia, we identified 26 SINE-R retroposons by PCR amplification. New SINE-R retroposons showed 89.7-96.6% sequence similarities with the sequence of the schizo-cDNA clone. The new retroposon sequences may cast light on the understanding of relationship between the retroviral elements and human diseases. According to the retrovirus hypothesis, recently proliferated retroviral elements in the human genome after separation of the human and chimpanzee could be implicated with the psychotic diseases (Crow, 1984). In the previous study, we identified two retroposons (HS307 and HS408) and four HERV-K LTRs (K-X7-3, K-X10-5, K-X13-1, and K-X13-2) in human Xq21.3 region by PCR approach using YAC clone panel (Kim et al., 1999b; Kim and Crow, 2001), as the Xq21.3 region has been known to be putatively linked to psychosis (Laval et al., 1998). We also identified such retroposons (HB1, HB2, HB4, HB5, HB6, HB7, and HB9) and HERV-K LTR elements (HKL-B7, HKL-B8, and HKL-B10) in human brain cDNA library (Kim et al., 2001; Kim and Lee, 2001), suggesting that the retroviral elements were actively transcribed in the human brain. The retroviral elements could be related to brain function implicated in the neuropsychiatric diseases. Those retroviral elements contained a hormone-responsive element (TGTTAT) and enhancer core (GTGCTAAG) sequences. SINE-R retroposons have a polypurine tract (PPT) that serves as a primer binding site for plus-strand DNA synthesis from retrovirally mediated reverse transcription (Ono et al., 1987). Akopov et al. (1998) noted that such sequences have the capacity to modify the expression of neighboring genes. In addition, a similar retrotransposable element to SINE-R.C2 (human-specific retroposon) has been reported as the cause of Fukuyama type muscular dystrophy, and this appears to be the first association of this class of elements with disease (Kobayashi et al., 1998). Taken together, the SINE-R retroposon and HERV-K LTR elements expressed in the brain tissues could be connected to brain diseases such as psychosis.

Multiple copies of SINE-R retroposons have been reported in humans and hominoid primates (Kim and Crow, 1999; 2000; Kim et al., 1999a, b, c; Kim et al., 2000a, b; Kim and Takenaka, 2001). Moreover, a retroviral sequence was reported from cDNA (schizo-cDNA) from postmortem tissue of the frontal cortex of an individual suffering from schizophrenia who had committed suicide at the age of 34 years (Yee et al.,

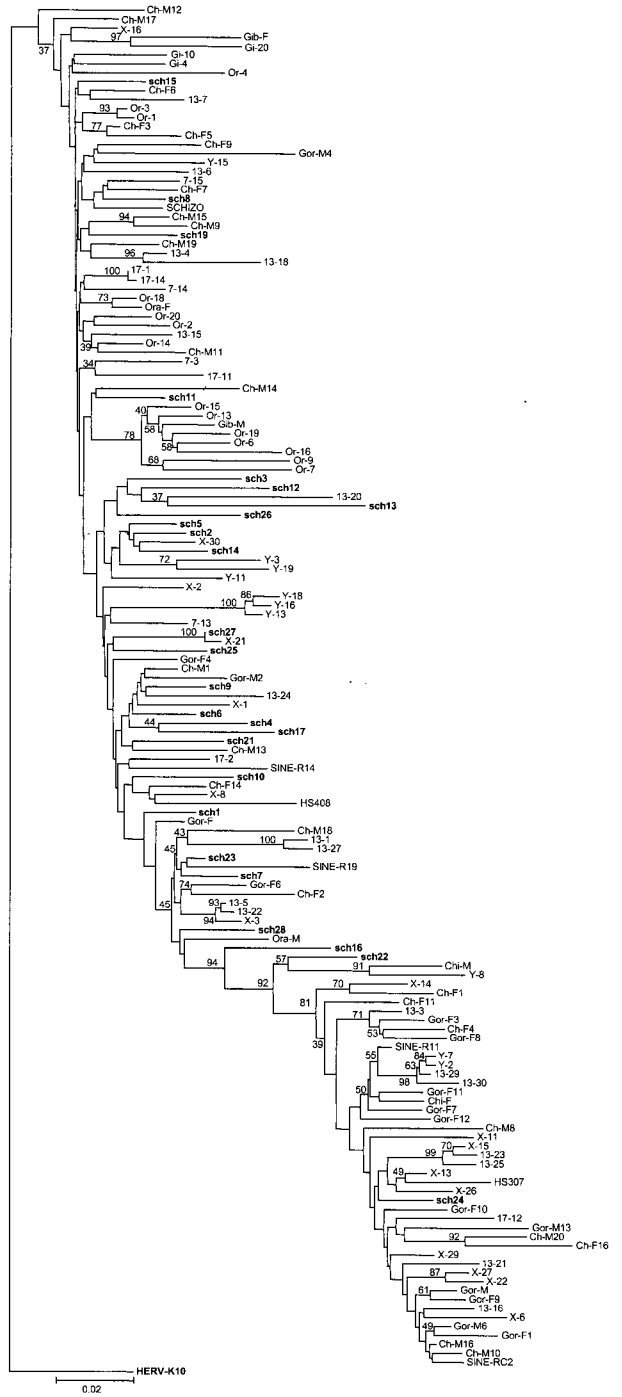

Fig. 1. Phylogenetic tree obtained by neighbor-joining method for the SINE-R retroposon family. HERV-K10 was used as outgroup. New SINE-R retroposons from schizophrenia are shown in bold letter. Branch lengths are proportional to the distances between the SINE-R retroposons. The values at branch-points indicate the percentage support for a particular node after 1000 bootstrap replications.

1998). In order to understand phylogenetic relationship, we analysed all the retroposons identified in schizophrenia together with hominoid-specific retroposons. As shown in Fig. 1, a phylogenetic tree from the data of the SINE-R retroposons indicates that the new SINE-R retroposons from schizophrenia have proliferated independently during hominid evolution. Such retroposons are randomly amplified in the genome of the schizophrenia. Those elements could freely change the genomic structure of the schizophrenia. Finding the new retroposon in schizophrenia give plentiful sources to analyze the candidate of the schizophrenia. In our previous study, we demonstrated that the SINE-R retroposons have been evolved parallel pattern in the course of hominoid primates (Kim et al., 1999c; Kim and Takenaka, 2001). Likewise, new SINE-R retroposons in schizophrenia showed such pattern in this research. To our knowledge, this is the first time the SINE-R retroposon family from the schizophrenia has been reported. Therefore, our sequence data from patients with schizophrenia indicate that the new SINE-R retroposons may be very useful for further study as potential leads on the understanding of neuropsychiatric diseases.

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