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Identification and Phylogeny of the Human Endogenous Retrovirus HERV-W LTR Family in Schizophrenia

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Abstract The long terminal repeat (LTR) elements of human endogenous retrovirus (HERV) have been found to be coexpressed with genes located nearby. It has been suggested that the LTR elements have contributed to the genetic variation of human genome connected to various diseases. Recently, HERV-W family was identified in the cerebrospinal fluids and brains of individuals with schizophrenia. Using genomic DNAs derived from schizophrenia, we performed PCR amplification and identified six HERV-W LTR elements. Those LTR elements showed a high degree of sequence similarity (87.7-99.5%) with HERV-W LTR (AF 072500). Sequence analysis of the HERV-W LTR elements revealed that clone W-sch1 showed identical sequence with the AC003014 (PAC clone RP1-290B4) derived from human Xq23. Clone W-sch2 was closely related to the AC007244-2 derived from human Y chromosome by phylogenetic analysis. Our data suggest that new HERV-W LTR elements in schizophrenia may be very useful for further studies to understand neuropsychiatric diseases.

Key words: HERV-W, LTR elements, Phylogeny, Schizophrenia

Introduction

Human endogenous retroviruses (HERVs) are footprints of ancient germ-cell retroviral infections [1]. Retroviral sequences may interact with cellular oncogenes [2] and long terminal repeat (LTR) elements have the capacity to exert a regulatory influence as promoters and enhancers of cellular genes. Comparative analysis of the HERV LTR elements in human genome could help us to understand the possible impact of HERVs on evolution and genome regulation.

Retroviral particles have been recovered from monocyte cultures from patients with multiple sclerosis [3] and virion-associated MSRV (multiple sclerosis associated retrovirus)-RNA has been reported in serum of patients with the disease [4]. Expression of MSRV sequences in normal placenta allowed the reconstruction of a 7.6 kb putative genomic retroviral RNA with RU5-gag-pol-env-U3R organization, with a polypurine binding site (PBS) showing similarity with avian retrovirus PBS used by tRNATrp [5]. Southern blot hybridizations using MSRV probes allowed characterization of a copy MSRV-related human endogenous retrovirus family named HERV-W [5]. We examined HERV-W *pol* and *env* gene sequences in human monochromosomes, and found multiple frameshift and termination codons by deletion/insertion or point mutation [6,7]. The HERV-W LTR elements were detected in hominoids, Old and New World monkeys, suggested that they have inserted in the primate genome approximately 55 million years ago [8]. The expression, structure and promoter activity of HERV-W LTR elements were examined in human cell lines [9]. Recently, the HERV-W family was identified in the cerebrospinal fluids and brains of individuals with schizophrenia [10]. Here we identified the HERV-W LTR elements in schizophrenia and phylogenetically analyzed with those sequences derived from GenBank database.

Materials and Methods

PCR amplification for HERV-W LTR elements

Genomic DNAs from blood samples of schizophrenia were used as a template for PCR amplification. New 416-bp LTR elements of HERV-W family were amplified by the primer pair HS47 (5′-TGGTCCATGTTTCTTACGGCT-3′, bases 127-147) and DS16 (5′-AAGATGGTGGTGAACCACTTC-3′, bases 521-541) from the HERV-W (GenBank, accession no. AF072500). The PCR conditions were used as described by Kim *et al.* [11] with an annealing temperature of 56°C.

Molecular cloning of PCR products

PCR products were separated on 2% agarose gel, purified with the QIAEX II gel extraction kit (Qiagen) and cloned

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into the T-khs307 vector [12]. The cloned DNA was isolated by the alkali lysis method using the High Pure plasmid isolation kit (Roche). Individual plasmid DNAs were screened for inserts by PCR using the original primers designed for the locus.

Determination of HERV-W LTR elements 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 [13]. Nucleotide sequences of HERV-W LTR elements were retrieved from the GenBank database with the aid of BLAST network server [14].

Nucleotide sequence accession numbers

The nucleotide sequences of six members of the HERV-W LTR elements reported in this paper have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the following accession numbers: clone W-sch1 (AB 071970), W-sch2 (AB071971), W-sch3 (AB071972), W-sch4 (AB071973), W-sch5 (AB071974), and W-sch6 (AB071975).

Results and Discussion

A retroviral sequence from postmortem tissue of the frontal cortex of an individual suffering from schizophrenia was reported [15]. Schizophrenia associated retrovirus (SZRV-1) was also identified from monozygotic twin pairs discordant for schizophrenia [16]. Recently, Karlsson et al. [10] reported the identification of retroviral sequences in cerebrospinal fluids obtained from individuals with recent-onset schizophrenia, and the differential transcriptional up-regulation of members of the HERV-W family of endogenous retroviruses in the postmortem frontal cortex of individuals with schizophrenia. Therefore, retroviruses could be strongly involved to the pathogenesis of schizophrenia as infectious agents. We performed PCR amplification and identified six HERV-W LTR elements using genomic DNAs derived from schizophrenia. They were aligned with the HERV-W LTR element (Fig. 1). One or two bp deletions or additions were notified in aligned sequences. Those LTR elements showed a high degree of sequence similarity (87.7-99.5%) with that of HERV-W LTR (AF072500) (Table 1). To understand the phylogenetic relationship among HERV-W LTR elements, we retrieved the LTRs from the GenBank database and analyzed them with new HERV-W LTR elements in schizophrenia. A phylogenetic tree obtained by the neighbor-joining method revealed that clone W-sch2 showed sister relationship with the AC007244-2 derived from human Y chromosome, while

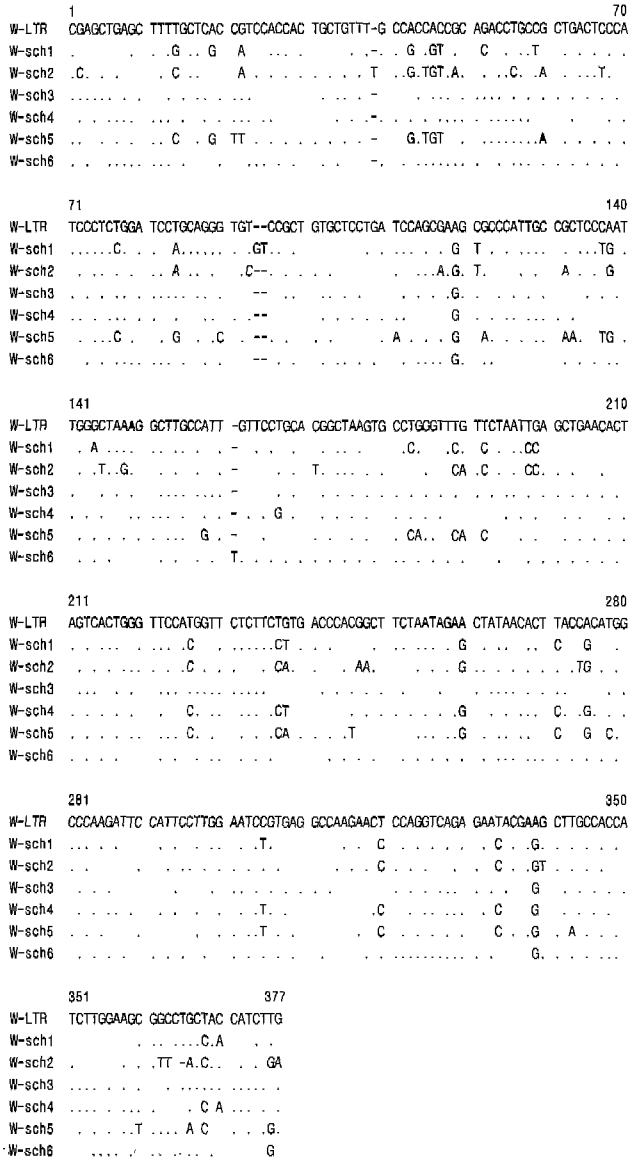

Fig. 1. Sequence alignments of the HERV-W LTR elements in schizophrenia.

Table 1. Percentage similarity of nucleotide sequences of HERV-W LTR elements in schizophrenia

	1	2	3	4	5	6	7
1. W-LTR	-						
2. W-sch1	90.9	-					
3. W-sch2	87.7	89.9	-				
4. W-sch3	99.5	91.5	88.2	-			
5. W-sch4	96.2	94.1	89.0	96.8	-		
6. W-sch5	88.7	92.0	89.3	89.3	91.2	-	
7. W-sch6	98.9	91.0	88.0	99.5	96.3	89.3	-

clones W-sch3, W-sch4, and W-sch6 were closely related to the AX000960, AF072504, and AF072506 from GenBank database (Fig. 2). Interestingly, clone W-sch1 showed iden

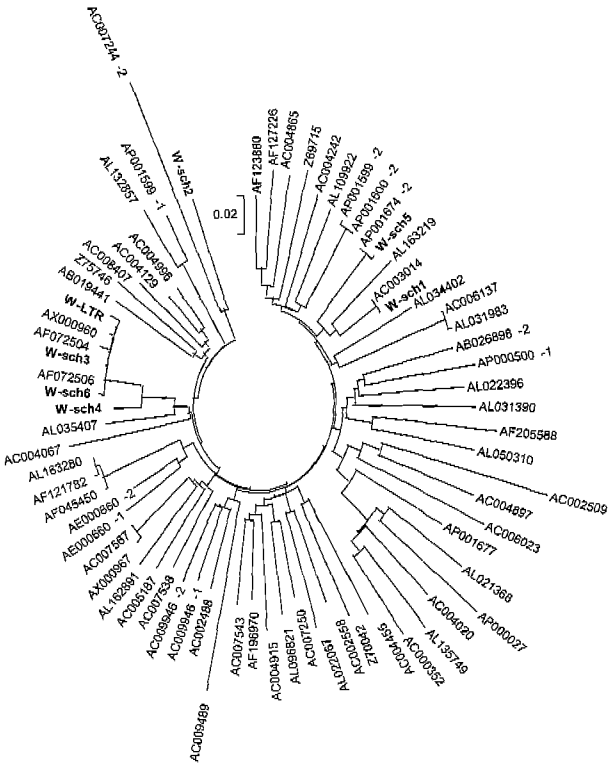

Fig. 2. Phylogenetic tree obtained by neighbor-joining method for the LTR elements of the HERV-W family in schizophrenia. Branch lengths are proportional to the distances between the taxa. New HERV-W LTR elements are shown in bold letter.

tical sequence with the AC003014 (PAC clone RP1-290B4) derived from human Xq23. Clone W-sch2 was also closely related to the AC007244-2 derived from human Y chromosome by phylogenetic analysis. Retroviral elements on sex chromosomes deserve further investigation in relation to the numerical abnormality for understanding neuropsychiatric diseases.

Akopov et al. [17] have noted that the LTR elements 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. Transcription of RNA homologous to members of the HERV-W family of retroviruses was found to be up-regulated differentially in the frontal cortex regions of brains obtained postmortem from individuals with schizophrenia [10]. The HERV-K-T47D-related LTR element has mediated polyadenylation of cellular transcripts [18]. Such phenomenon was very recently demonstrated in nucleosomal binding protein NSBP1 in Xq13.3 [19]. The retroviral LTR element (DQLTR3) could enter into the human genome as human-specific form [20]. This type of the retroviral elements also induced alternative splicing in the human leptin receptor [21]. The solitary HERV LTR elements showed that they retained detectable activity in human carcinoma cells, and could direct the transcription in both orientations relative to the reporter gene [22]. In this report, our new sequence data of the HERV-W LTR elements in schizophrenia may be of great use in future studies for understanding neuropsychiatric diseases.

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