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Identification and phylogenetic analysis of the human endogenous retrovirus HERV-W *pol* in cDNA library of human fetal brain

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Abstract

A human endogenous retroviral family (HERV-W) has recently been described that is related to multiple sclerosis-associated retrovirus (MSRV) sequences that have been identified in particles recovered from monocyte cultures from patients with multiple sclerosis. Two *pol* fragments (HWP-FB10 and HWP-FB12) of HERV-W family were identified and analysed by the PCR approach with cDNA library of human fetal brain. They showed 89 percent nucleotide sequence similarity with that of the HERV-W (accession no. AF009668). Deletion/insertion or point mutation in the coding region of the *pol* fragments from human fetal brain resulted in amino acid frameshift that induced a mutated protein. Phylogenetic analysis of the HERV-W family from GenBank database indicates that the HWP-FB10 is very closely related to the AC000064 derived from human chromosome 7q21-q22. Further studies on the genetic relationship with neighbouring genes and functional role of these new HERV-W *pol* sequences are indicated.

Key words – cDNA library; Fetal brain; HERV-W; *pol* fragments; Phylogeny

Introduction

It has been suggested that endogenous retroviruses and retroposons have played a role in influencing the functional organization of the human genome [5,21]. Multiple copy numbers of the retroviral elements and SINE-R retroposons are detected in human genome [9,10, 12,13] and various cancer cell lines [26]. Full length retroviral sequences may interact with cellular oncogenes [22]. Retroviral long terminal repeat (LTR) sequences have the capacity to exert a regulatory influence as promoters and enhancers of cellular genes [19]. The retrotransposition process implicates reverse transcription of RNA intermediate by reverse transcriptase. The nucleotide sequences encoding this enzyme are conserved [27]. Some retroviral sites of integration may represent tandem duplications and are non-functional, others appear to be transcriptionally active [17,20].

Retroviral particles have been recovered from monocyte cultures from patients with multiple sclerosis [18] 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 7.6kb 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 [2]. Another recent study has associated transposition of a MSRV-like sequence with schizophrenia, using representational difference analysis on monozygotic twins discordant for schizophrenia [3]. This new

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family of endogenous retroviral sequences has been named HERV-W.

Recently, chromosomal distributions of the HERV-W *gag, pro,* and *env*-related sequences by Southern blot analysis were reported [24]. The various copy numbers of the *gag, pro,* and *env* genes were tandem dispersed among human chromosomes. They were integrated into the genome of the catarrhines more than 25 million years ago [15,23]. The *pol* and *env* gene sequences of the HERV-W family from human monochromosomes were identified [7,14]. Here the *pol* gene family of the HERV-W was identified in cDNA library of the human fetal brain and phylogenetically analysed with those of the GenBank database.

Materials and Methods

PCR amplification of HERV-W family

The cDNA synthesized from mRNA of human fetal brain (Clontech) was used as a template for PCR amplification. New 693-bp *pol* fragments of HERV-W family were amplified by the primer pair; HS48 (5-ACTACCTGTGGCTACAAGGTT-3, bases 1030-1050) and HY78 (5-AAGTGCGCAGTCTCAGCA-3, bases 1720-1703) from the HERV-W (GenBank, accession no. AF009668). The PCR conditions were previously described in Kim et al. [11] with an annealing temperature of 56°C.

Molecular cloning of PCR product

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 [8]. The cloned DNA was isolated by the alkali lysis method using the high pure plasmid isolation kit (Roche).

DNA sequencing 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 from the GCG program (University of Wisconsin). Neighbor-joining phylogenetic analysis was performed with the MEGA program (version 1.01) [16]. Statistical significance evaluation of the branching pattern for bootstrap values was performed with 100 replications. Nucleotide sequences of HERV-W family were retrieved from the GenBank database by BLAST program [1].

Nucleotide sequence accession numbers

The nucleotide sequences of two new members of the HERV-W reported in this study will be appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the following accession numbers: HWP-FB10 (AB063618) and HWP-FB12 (AB063619).

Results and Discussion

The chromosomal localization and nucleotide sequences of the HERV-W *pol* family were analysed using human monochromosomal DNA panel in previous study [7]. Moreover, such family was also identified on several human chromosomes using BLAST search from the GenBank database. Table 1 shows the summary of the HERV-W family. Except for human chromosomes 9, 16, 17, 18, and 22, the HERV-W family was detected on all chromosomes. In this study, two *pol* fragments (HWP-FB10 and HWP-FB12) belonging to the HERV-W family were identified from cDNA library of the human fetal brain (Fig. 1). They showed 89.3 percent sequence similarity with that of HERV-W (AF009668). Interestingly, the HWP-FB10 showed 99.8% sequence similarity with that of the AC000064 (BAC clone RG083M05) derived from human chromosome 7q21-q22. Only a transition (T to C) has occurred between the sequences. According to the BLAST search, several genes, STEAP (six transmembrane epithelial antigen of the prostate), DLX6 (distal-less

Table 1. Chromosomal localization of *pol* gene of the HERV-W family

Chromosome No.	Accession no. of the *pol* gene	
	Monochromosome data[1]	GenBank data
1		AL445991, AL122001, AF135487
2	AB056481, AB056482	AF135487
3	AB056483, AB056484, AB056485	AF135487, AP000500
4	AB056486, AB056487	AC005187
5	AB056488, AB056489	AC016617
6	AB056490, AB056491	AL121969, AL031775, AL109922, AL031390,
7		AL139090, AL355352, AL360227, AL391416
8	AB056492	AC000064, AC007567
9	AB056493	
10		
11	AB056494	
12	AB056495	
13	AB056496	AC078963
14	AB056497	
15	AB056498	AL121579
16	AB056499	
17		
18		
19		AC024563
20	AB056500	AL035663, AL360078
21	AB056501	AF121782, AL163280
22		
X	AB056502, AB056503	AL157698, AL162912, Z83850, AL008629
Y	AB056504	AC007244

[1]From Kim [7]

homeo box 6), CYP3A5 (cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 5), and SRPK2 (SFRS protein kinase 2), were localized on human chromosome 7q21-q22. Therefore, considering both sequences of the HWP-FB10 and AC000064 are identical, the HWP-FB10 sequences could be of great interest to analyse continuously as implication of the neighbouring gene connection. In an analysis of the HWP-FB12 sequences, the HWP-FB12 did not show high level of the sequence similarity with that of the HERV-W family indicated in Table 1. The data imply that the HWP-FB12 sequences were not yet identified in GenBank database, indicating that the HWP-FB12 sequences belonged to the HERV-W family as a new member. Further studies of the chromosomal localization and characterization are needed using the HWP-FB12 clone. We were also interested in analysing the evolutionary pattern (synonymous/nonsynonymous substitutions). To see such results, we analysed the putative amino acid sequences of the HWP-FB10 and HWP-FB12, indicating that translation of the both *pol* fragments showed multiple frameshift and termination codon by deletion/insertion or point mutation (Fig. 1). This suggests that the HERV-W family in human fetal brain may not active as a provirus.

To understand the evolutionary relationship within the HERV-W family on human chromosomes, a phylogenetic tree was constructed using the neighbor-joining method with the HERV-W family obtained from the GenBank

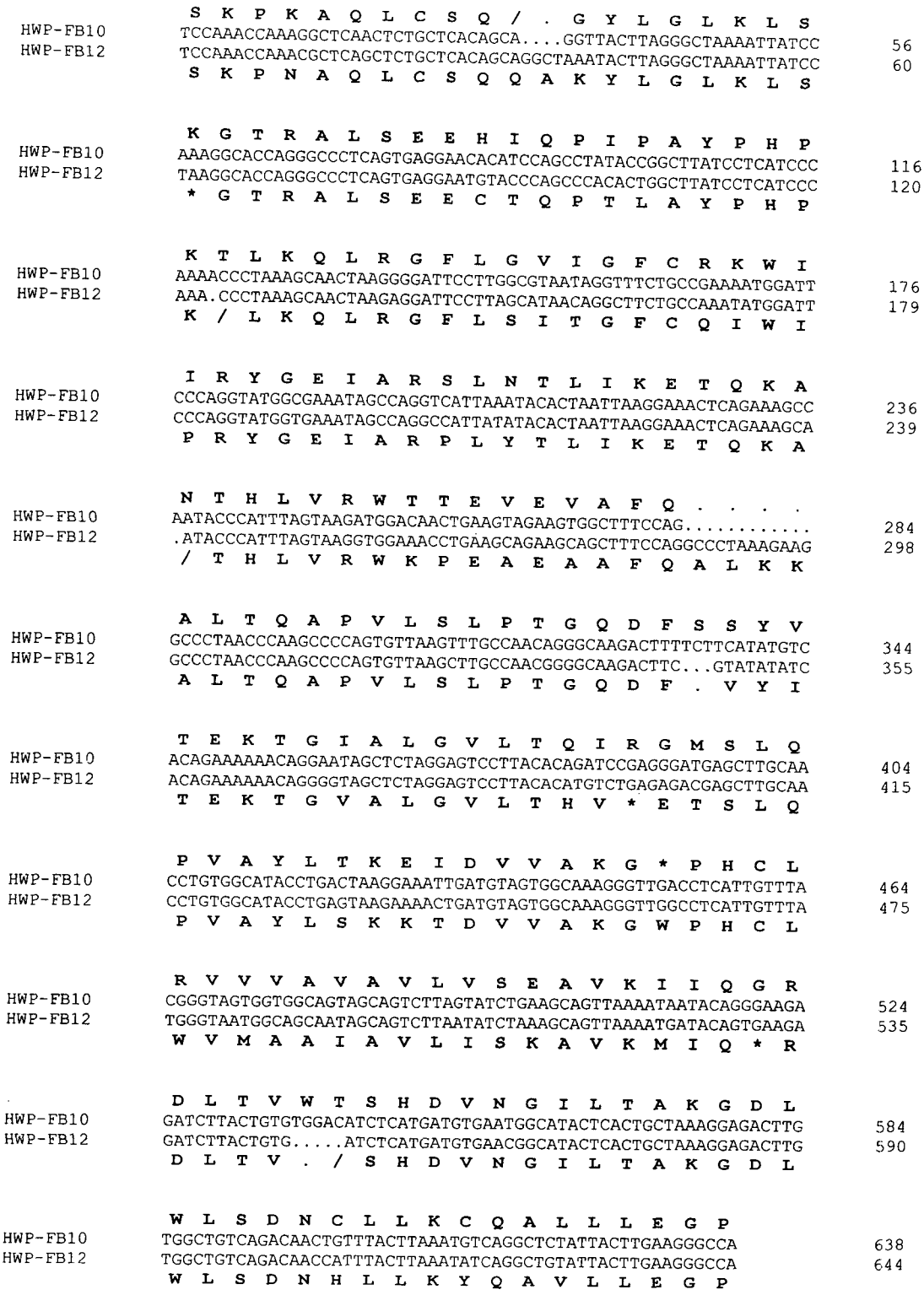

Fig. 1. Sequences of the HWP-FB10 and HWP-FB12 from the cDNA library of the human fetal brain together with the translation of the *pol* fragments. Amino acids are aligned with the second nucleotide of each codon. Frameshifts are indicated by a slash and stop codons by an asterisk.

database. As shown in Fig. 2, the HWP-FB10 showed sister relationship with the AC000064 (chromosome 7q21 - q22), while the HWP-FB12 clustered with the HWPX-1 (chromosome 1), AL162912 (chromosome Xq13.3 - q21.2), and AL445991 (chromosome 1). Both two clones (HWP-FB10 and HWP-FB12) belonged to the large group that as large branch length, indicating that the HWP-FB10 and HWP-FB12 clones could be integrated into the human genome at least 5 million years ago. Wimmer et al. [25] demonstrated the transposition of chromosome 1 sequences to chromosome Y before separating between humans and chimpanzees (5 million years ago) by FISH

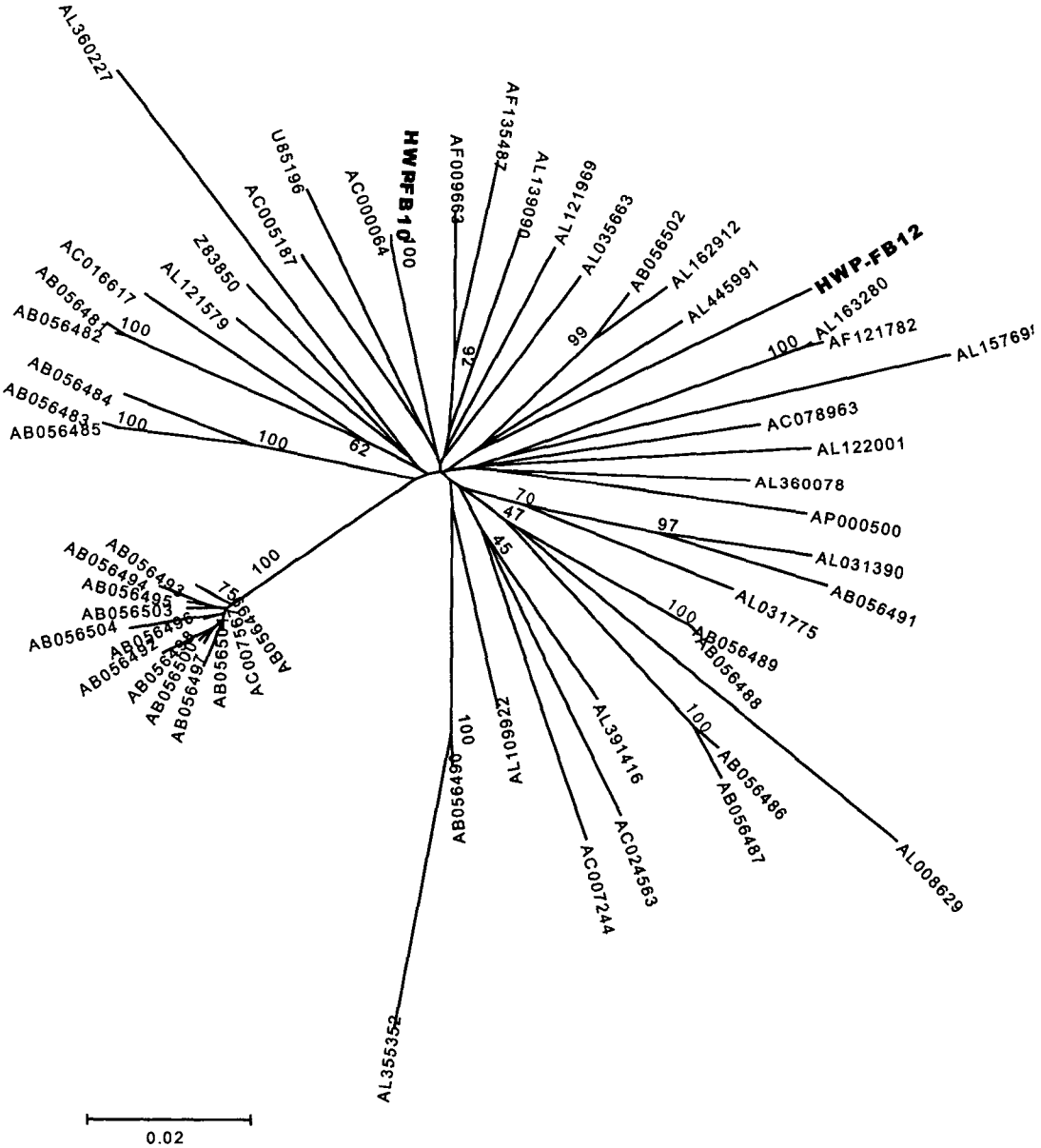

Fig. 2. Phylogenetic tree obtained by neighbor-joining method for the *pol* fragments of the HERV-W family. Branch lengths are proportional to the distances between the taxa. The values at branch points indicate the percentage support for a particular node after 100 bootstrap replications. New HWP-FB10 and HWP-FB12 belonging to the HERV-W family from the cDNA library of the human fetal brain in this report are indicated as bold letters.

analysis. This synapomorphic trait provided clear evidence for a human-chimpanzee clade independent of DNA sequences. Recently, the HERV-W sequences were identified in cerebrospinal fluids obtained from individuals with recent-onset schizophrenia [6]. He also reported the differential transcriptional up-regulation of members of the HERV-W family of endogenous retroviruses in the postmortem frontal cortex of individuals with schizophrenia. Deb-Rinker et al. [3] also identified SZRV (schizophrenia retrovirus) from the schizophrenia patients, which belonged to the HERV-W family. The HWP-FB10 and HWP-FB12 clones belonging to the HERV-W family were transcribed in human fetal brain, suggesting that they represent a source of genetic variation connected to brain diseases such as psychosis. Therefore, the present data could be useful for further study in relation to neuropsychiatric diseases in human brain.

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초록 : 인간태아의 뇌로부터 유래된 cDNA library에서 내생레트로바이러스 HERV-W pol 유전자의 동정과 계통

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인간 내생 레트로바이러스 HERV-W는 다발성 경화증 환자로부터 탐지된 MSRV와 연루되어 있다. 인간 태아의 뇌로부터 유래된 cDNA library를 이용하여 PCR법으로 2개의 HERV-W 패밀리(HWP-FB10과 HWP-FB12)를 동정하고 분석하였다. 그들은 HERV-W (accession no. AF009668)와 89%의 염기서열의 유사성을 보였다. *Pol* 유전자를 아미노산의 서열로 분석해 본 결과 점돌연변이 또는 삽입/결실로 말미암아 frameshift 및 종결코돈을 나타내었다. 유전자정보의 데이터베이스를 이용하여 HERV-W 패밀리간의 분자계통분류도를 작성해 본 결과 HWP-FB10은 인간의 염색체 7q21-22로부터 유래된 AC000064와 매우 가깝게 관련되어 있음을 시사하였다. 이들의 새로운 HERV-W *pol* 패밀리가 이웃하는 어떤 유전자와 상호 연결되어 있으며, 어떠한 기능을 수행하는지에 대한 전망에 대해 토의하였다.