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Expression and Identification of HERV-W Family in Japanese Monkeys (*Macaca fuscata*)

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ABSTRACT—We investigated structural genes (*gag*, *pol*, *env*) of HERV-W family in the *Macaca fuscata* (Japanese monkey). Those genes are expressed in various tissues (testis, prostate, kidney, cerebellum, thymus, pancreas, intestine, stomach, ovary) of the Japanese monkey in RT-PCR and sequencing analyses. Nine clones for *gag*, thirty-one clones for *pol* and thirty-four clones for *env* fragments of the HERV-W family in monkey tissues were identified and analyzed. These clones showed a high degree of sequence similarity, 82.2–84.7% for *gag*, 88.4–91.7% for *pol*, and 90.8–95.4% for *env*, to those of HERV-W family. Translation to amino acids in all clones derived from the monkey indicated that they showed multiple interruptions of frameshifts and termination codons by deletion/insertion or point mutation. Identical sequences from different tissues of the monkey were found in *env* and *pol* clones of the HERV-W family.

Key words: Human endogenous retrovirus W (HERV-W) family, Japanese monkey, expression, phylogeny

INTRODUCTION

Retroviruses are enveloped plus strand RNA viruses with a mode of replication in which the RNA genome is reverse transcribed into DNA and integrated into the genome of host as a provirus. The conversion from RNA to DNA is acquired by the virus-encoded enzyme reverse transcriptase (RT). The large numbers of endogenous retroviruses have been amplified in primates during evolution by repeated reintegration of reverse-transcribed mRNA into the DNA of germline cells. Human endogenous retroviruses (HERV) have been detected by using their partial homology to animal retroviruses either by low stringency hybridization with probes derived from murine or primate proviral DNAs (Leib-Mosch *et al.*, 1986). Approximately 8% of human genome is comprised of human endogenous retrovirus gene sequences and long terminal repeat (LTR) –like elements, as well as thousand of HERV-like elements (International Human Genome Sequencing Consortium, 2001). Most HERV families were amplified in the germ line 35–45 million years ago. Previously, phylogenetic significance and evolutionary studies suggested that HERVs mostly inserted into the genome early in the primate evolution (Costas, 2001; de

Parseval *et al.*, 2001; Hughes and Coffin, 2001; Kim *et al.*, 2002). To date, at least 22 independent HERV families within human genome were identified (Tristem, 2000).

Expression of HERVs can influence the outcome of infections in different ways that can be either beneficial or detrimental to the host. Most HERV elements are highly defective with large deletions, stop codons and frameshifts in the open reading frames (ORFs). A function of the multi copy families, scattered throughout the genome, has been reported regulatory functions on the gene expression of nearby located genes (Akopov *et al.*, 1998). Long terminal repeat (LTR) sequences have the capacity to exert a regulatory influence as promoters and enhancers of cellular genes (Schon *et al.*, 2001). Likewise, the gene expression could be regulated by its neighboring retroviral sequences, HERV-W family (Alliel *et al.*, 2002). A retroviral element that carries a primer binding site (PBS) homologous to the 3' end of a tryptophan tRNA is named HERV-W (Blond *et al.*, 1999). The HERV-W family consists of an estimated 30 to 100 provirus copies per haploid human genome. Phylogenetic relationship revealed that the HERV-W family is related to ERV-9 and RTLH families (Boeke and Stoye, 1997). *Gag* and *pol* open reading frames (ORFs) of the HERV-W family were interfered with frameshifts and stop codons by deletions/ insertions, whereas a complete *env* ORF encoding an envelope was found on the cl.PH74 placental cDNA clone and genomic RG083M05 clone (Blond *et*

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al., 1999). It was suggested that HERV-W has a chimeric genome structure as following homologies with murine type C and simian type D retroviruses within the *pol* and *env* genes, respectively. The HERV-W has been one of the most broadly investigated HERVs during the last few years. The HERV-W on chromosome 7 has 100% identity to syncytin cDNA, which is expressed in the placenta, specifically in the syncytiotrophoblast layer (Alliel *et al.*, 1998; Mi *et al.*, 2000). This observation indicated that HERV-W family was involved to human placental development. With the advances of the functional study, the products of the HERV-W *env* gene is highly fusogenic membrane glycoprotein that induces the formation of syncytia on interaction with the type D mammalian retrovirus receptor (Blond *et al.*, 2000) and suggested direct role in human trophoblast cell fusion and differentiation (Frendo *et al.*, 2003). Recently, the HERV-W gene product blocked infection by any exogenous retrovirus, suggesting that expressed HERV genes could have beneficial functions to the host (Ponferrada *et al.*, 2003). The possibility that they have relevance to pathogenic capacity could be subjected to change in primate evolution and could be associated with variation between different tissues and with gene expression. In this study, we investigated expression of the HERV-W family in various tissues from the Japanese monkey.

MATERIALS AND METHODS

RNA samples and RT-PCR analysis

Total RNA from various tissues (testis, prostate, kidney, cerebellum, thymus, pancreas, intestine, stomach, ovary) in the Japanese monkey (*Macaca fuscata*) was extracted by TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). Polyadenylated mRNA was purified using the Poly(A)Tract mRNA isolation system (Promega, Madison, WI, USA). Using the pure mRNA only, expression patterns of the structural genes (*gag*, *pol*, *env*) of the HERV-W were examined by the Titan One Tube RT-PCR System (Roche, Indianapolis, IN, USA). Using reverse transcription - polymerase chain reaction (RT-PCR) approach, the HERV-W structural genes were identified from the mRNA of monkey tissues. As a standard control, G3PDH was amplified by the primers GPH-S (5'-CAAAGTTGTCATGGATGACC-3', bases 31721-31740) and GPH-AS (5'-CCATGGAGAAGGCTGGGG-3', bases 31898-31915) from the human G3PDH (GenBank accession no. AC068657). The HERV-W *env* fragments were amplified by the primer pair HS46 (5'-TCCCTGTACCTGAA-CAATGG-3', bases 1360-1379) and HY76 (5'-CTTTCAGCGGT-TAGCAAGTC-3', bases 1920-1939) from the HERV-W (GenBank, accession no. AF072506). The HERV-W *pol* fragments were amplified by the primer pair HS48 (5'-ACTACCTGTGGCTACAAGGTT-3' bases 1030-1050) and HY78 (5'-AAGTGCAGCTCTCAGCA-3' bases 1703-1720) from the HERV-W (GenBank, accession no. AF009668). The HERV-W *gag* fragments were amplified by the primer pair JM76 (5'-AAACGTTCCCGCAAGACAA-3' bases 611-630) and JM28 (5'-GGTGTTTTACAAGGGTTAGG-3' bases 1151-1170) from the HERV-W (GenBank, accession no. AF072500). The RT-PCR conditions followed were standard protocol of Titan One Tube RT-PCR System (Roche, Indianapolis, IN, USA) with an annealing temperature of 56°C.

Molecular cloning of RT-PCR products

RT-PCR products were electrophoresed on 2% agarose gel,

purified with the QIAEX II gel extraction kit (QIAGEN, Chatsworth, CA, USA) and cloned into the pGEM T-easy vector (Promega, Madison, WI). The cloned DNA was isolated by the alkaline lysis method using the high pure plasmid isolation kit (BIONEER, Daejeon, KOREA).

Sequencing and data analyses

Individual plasmid DNA was screened for inserts by PCR. 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, Foster City, CA, USA). Nucleotide sequence analyses were performed using GAP, PILEUP, and PRETTY from the GCG package (University of Wisconsin, Madison, WI, USA). Phylogenetic analyses using neighbor-joining and maximum parsimony methods were performed with the MEGA program (Kumar *et al.*, 2001). Statistical significance evaluation of the branching pattern was performed with 100 replications. Nucleotide sequences of the HERV-W families were retrieved from the GenBank database with the aid of the BLAST network server (Altschul *et al.*, 1997).

Nucleotide sequence accession numbers

In this study, identified all clones contained HERV-W families have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the following accession numbers: AB126132 – AB126165 (34 clones for *env*), AB126101 – AB126131 (31 clones for *pol*), AB126092 – AB126100 (9 clones for *gag*) from nine tissues of Japanese monkey.

RESULTS AND DISCUSSION

The HERV-W *env* gene (syncytin ORF) on chromosome 7q21 (GenBank accession no. AC000064) was expressed in human placenta and testis with two transcripts of 4 and 8 kb in Northern blot analysis (Mi *et al.*, 2000). The syncytin could mediate placental cytotrophoblast fusion connected to placental development. As shown in Fig. 1, the *env* gene expression was detected in various tissues, testis, prostate, kidney, cerebellum, thymus, pancreas, intestine, stomach, and ovary of the Japanese monkey by RT-PCR analysis, whereas in case of *gag* and *pol* gene, their patterns were detected as tissue specific. The *gag* gene was expressed in testis and placenta only, while the *pol* gene was expressed in testis, prostate, thymus, pancreas, intestine, stomach, and ovary. In order to confirm sample RNAs, we performed PCR amplification without RT reaction with pure mRNA samples, indicating that the prepared mRNA samples from the monkey tissue did not contain the genomic DNA (Data not shown).

Recently BLAST search for HERV-W family from DDBJ/EMBL/GenBank databases showed 140 sequences, representing 39 HERV-W proviruses, 40 full-length HERV-W retroposons, and 61 truncated HERV-W retrosequences (Costas, 2002). The number of identified HERV-W related fragments, at least 70 copies for *gag*, 30 copies for *env* per haploid genome, is correlated with the increase in complexity from *env* to *gag* and *pro* regions previously described (Voisset *et al.*, 2000). We previously isolated fifteen *env* and twenty-four *pol* members of the HERV-W family using human monochromosomal DNAs and suggested that ret-

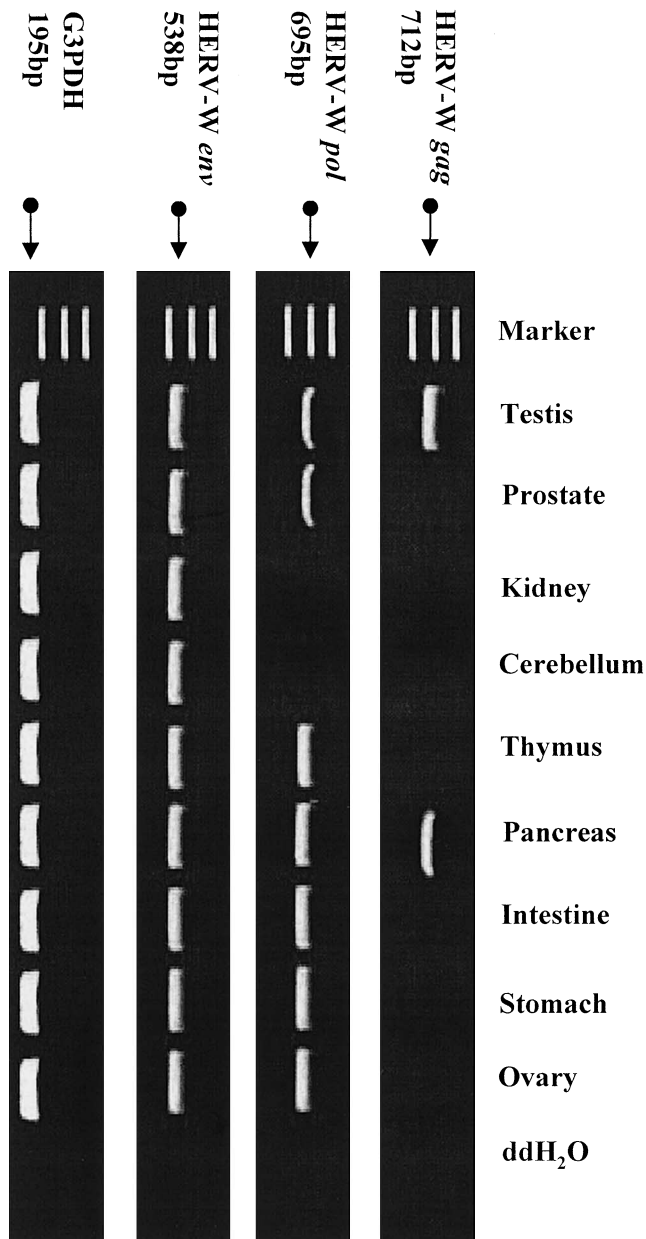


Fig. 1. RT-PCR analysis of mRNA for the expression of HERV-W structural genes from various tissues of the Japanese monkey.

rotransposition or chromosomal translocation had occurred during hominid evolution (Kim and Lee, 2001; Kim, 2001). We already identified expressed HERV-W related sequences, 17 sequences for *gag*, 53 sequences for *pol*, 95 sequences for *env* from 12 different human tissues by RT-PCR (manuscript submitted). This research motivated us to examine in monkey tissues. In the present study, we identified 9 *gag* sequences, 31 *pol* sequences, and 34 *env* sequences of the HERV-W in various monkey tissues (Table 1). The *env* sequences expressed from monkey tissues showed high degree of sequence similarity (90.8–95.4%) to that of human HERV-W (AF072506). Deduced amino acid sequences of 34 clones from various tissues of the monkey showed translation interruptions by point

mutations or deletion/insertions (Fig 2). The *gag* sequences showed 82.2–84.7% similarity to that of HERV-W (AF072500), while the *pol* sequences showed 88.4–91.7% similarity to that of human HERV-W (AF009668). Putative amino acid sequences of *gag* and *pol* genes also exhibited multiple frameshifts and termination codons caused by deletion/insertions or point mutations in all clones identified in this study (Fig 3). The data suggest that HERV-W structural genes have been scattered into monkey genome during primate evolution and those members could indirectly influence to the functional capacity in monkey tissues.

The HERV-W family has been phylogenetically analyzed from the databases, indicating that they were divided into three different subfamilies of the HERV-W in the human genome (Costas, 2002). The average divergence between sequences for each of the subfamilies indicated that most of the HERV-W elements were inserted within the genome during a short period of evolutionary time (Costas, 2002). In order to understand the relationship within the HERV-W family in human and monkey, we made phylogenetic analyses of cDNA sequences for *gag*, *pol*, and *env* genes of the HERV-W family. Neighbor-joining trees based on 695-nt for *pol*, and 538-nt for *env* fragments of HERV-W from monkey tissues were constructed, respectively (Figs. 4A, 5A). The topology was also supported by the maximum parsimony trees (Figs. 4B, 5B). We used the gene sequence of ERV-9 as an outgroup. Among HERV-W *env* clones derived from the monkey tissues, JM-WE1-3 (testis), JM-WE2-1 (prostate), JM-WE3-1 (kidney), JM-WE5-1 (thymus), JM-WE6-2 (pancreas), JM-WE7-5 (intestine), and JM-WE9-1 (ovary) belonging to E-1 group showed 100% sequence similarity each other, although they were derived from different tissues. These phenomena were also found in E-2 [JM-WE7-4 (intestine), JM-WE9-3 (ovary), and JM-WE8-1 (stomach)] and E-3 [JM-WE6-9 (pancreas) and JM-WE8-9 (stomach)] groups (Fig. 5). Likewise, the HERV-W *pol* clones in Japanese monkey also showed several identical sequences each other in different monkey tissues, such as P-1 [JM-WP8-7 (stomach) and JM-WP9-1 (ovary)], P2 [JM-WP2-11 (prostate) and JM-WP5-3 (thymus)], P-3 [JM-WP2-2 (prostate) and JM-WP5-6 (thymus)], and P-4 [JM-WP6-6 (pancreas), JM-WP7-1 (intestine), JM-WP8-4 (stomach), and JM-WP9-2 (ovary)] groups (Fig. 4). It seems that HERV-W genes are actively expressed in various monkey tissues. Clustering pattern of the HERV-W clones in humans and monkeys allowed us to speculate that they have continuously been evolved randomly during primate radiation.

To our knowledge, this study is first report that HERV-W structural genes are investigated the tissue distribution of non-human primate and analyzed their relationships phylogenetically between human and monkey. The *gag* and *pol* genes of HERV-W were expressed specifically in multiple monkey tissues (Fig 1). Several studies on HERV transcription have been performed and revealed preferential expression of the HERV families in the human tissues and abnormal tissues such as tumor. Accordingly, there has

Table 1. HERV-W elements identified from Japanese monkey tissues

Japanese monkey tissues	W-gag clones	W-pol clones	W-env clones
1. Testis	JM-WG1-1, JM-WG1-2, JM-WG1-4, JM-WG1-5, JM-WG1-8, JM-WG1-9	JM-WP1-2, JM-WP1-4, JM-WP1-10, JM-WP1-12	JM-WE1-1, JM-WE1-3, JM-WE1-4, JM-WE1-10
2. Prostate		JM-WP2-2, JM-WP2-11	JM-WE2-1, JM-WE2-4, JM-WE2-5, JM-WE2-6, JM-WE2-8
3. Kidney			JM-WE3-1, JM-WE3-3
4. Cerebellum			JM-WE4-1, JM-WE4-2
5. Thymus		JM-WP5-1, JM-WP5-2, JM-WP5-3, JM-WP5-5, JM-WP5-6, JM-WP5-7, JM-WP5-9	JM-WE5-1, JM-WE5-3, JM-WE5-4
6. Pancreas	JM-WG6-2, JM-WG6-3, JM-WG6-4	JM-WP6-2, JM-WP6-3, JM-WP6-4, JM-WP6-5, JM-WP6-6, JM-WP6-7, JM-WP6-8, JM-WP6-9	JM-WE6-2, JM-WE6-6, JM-WE6-9
7. Intestine		JM-WP7-1, JM-WP7-2, JM-WP7-5, JM-WP7-8	JM-WE7-1, JM-WE7-2, JM-WE7-3, JM-WE7-4, JM-WE7-5, JM-WE7-8
8. Stomach		JM-WP8-1, JM-WP8-4, JM-WP8-7	JM-WE8-1, JM-WE8-2, JM-WE8-4, JM-WE8-7, JM-WE8-8, JM-WE8-9
9. Ovary		JM-WP9-1, JM-WP9-2, JM-WP9-3	JM-WE9-1, JM-WE9-3, JM-WE9-6

CONSENSUS	NNFSTEINAT	SVLVGPLVSN	LEITHSNTLT	CVKFNNTIDT	/TNSQCIKRV	TPPTRIVCLP	SGIFFVCGTS	AY-CLNGSSE	SMCFLSFLAP	PVTIYTEQVL
HERV-W	-----T-	-----	-----	-----S-TY-	-----	-----Q	-----	-----R	-----V-	-----M-----D ¹⁰⁰
JM-WE2-1	-----*TI	-----	-----	-----CS-LL*	-----G*	-----QM	-----QKSL/-	-----S	-----	-----D
JM-WE2-3	-----	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----
JM-WE2-4	-----*TI	-----	-----	-----CS-LL*	-----G*	-----QM	-----QKSL/-	-----S	-----V	-----SM-----D
JM-WE2-10	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE3-1	-----	-----	-----X	-----	-----	-----	-----	-----	-----	-----D
JM-WE3-4	-----	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----
JM-WE3-5	-----T	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----
JM-WE3-6	-----	-----	-----S	-----	-----	-----	-----	-----	-----	-----
JM-WE3-8	-----S	-----V-A	-----	-----Y-CS-T	-----A	-----C	-----/	-----V-G	-----V	-----M-----D
JM-WE4-1	-----	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----
JM-WE4-3	-----T	-----	-----R	-----N-SS	-----	-----	-----	-----H	-----V	-----M-----D
JM-WE5-1	-----	-----X	-----	-----S	-----V	-----	-----	-----	-----	-----
JM-WE5-2	-----	-----	-----	-----X	-----A	-----	-----	-----	-----	-----P-H
JM-WE6-1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE6-3	-----T	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----L
JM-WE6-4	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE7-2	-----	-----	-----	-----	-----	-----	-----	-----H	-----V	-----M-----D
JM-WE7-6	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE7-9	-----T	-----	-----R	-----N-SS	-----	-----S-QT-L	-----	-----R-D	-----M	-----M-----D
JM-WE8-1	-----T	-----	-----	-----Y-S-A	-----	-----	-----	-----	-----	-----M-----D
JM-WE8-2	-----	-----	-----	-----A	-----	-----	-----/	-----V-G	-----V	-----M-----D
JM-WE8-3	-----S	-----V-A	-----	-----Y-CS-T	-----A	-----C	-----/	-----V-G	-----	-----M-----D
JM-WE8-4	-----	-----	-----	-----A	-----	-----	-----	-----	-----	-----
JM-WE8-5	-----	-----	-----X	-----	-----	-----C	-----/	-----V-G	-----	-----A
JM-WE8-8	-----S	-----V-MA	-----X	-----Y-CS-T	-----A	-----C	-----/	-----V-G	-----	-----A
JM-WE9-1	-----	-----	-----X	-----A	-----	-----	-----	-----	-----	-----
JM-WE9-2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE9-4	-----	-----	-----	-----	-----	-----	-----	-----H	-----V	-----M-----D
JM-WE9-7	-----T	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----
JM-WE9-8	-----	-----	-----	-----A	-----	-----	-----	-----	-----	-----
JM-WE9-9	-----T	-----	-----R	-----N-SS	-----	-----	-----	-----	-----	-----
JM-WE10-1	-----	-----	-----X	-----	-----	-----	-----	-----	-----	-----
JM-WE10-3	-----	-----	-----	-----A	-----	-----	-----	-----	-----	-----
JM-WE10-6	-----	-----	-----	-----A	-----	-----	-----	-----	-----	-----
CONSENSUS	YNHVVPKPHN	KRV/ILPFVI	GAGVLGLGT	GIGGITPTQ	FYYKLSQELN	GDMERVANSI	V-TLQGG/LN	/LAAVVLQNG	RAL	
HERV-W	-----SY-S-R	-----P	-----A	-----S	-----	-----D	-----D	-----S	-----R	-----R ¹⁸³
JM-WE2-1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE2-3	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE2-4	-----	-----	-----	-----	-----P	-----Q-D	-----/D	-----F	-----*	-----S
JM-WE2-10	-----C	-----P	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE3-1	NSY	-----P	-----	-----S	-----	-----N-Q-TD	-----D	-----S	-----*	-----
JM-WE3-4	-----	-----	-----R	-----	-----	-----	-----	-----	-----	-----
JM-WE3-5	-----	-----	-----R	-----	-----	-----	-----	-----	-----	-----
JM-WE3-6	-----	-----	-----	-----S	-----	-----	-----	-----	-----	-----
JM-WE3-8	-----Y-H-R	-----P	-----R	-----	-----	-----	-----	-----	-----	-----
JM-WE4-1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE4-3	-----Y-H-R	-----P	-----R	-----	-----	-----V	-----	-----	-----	-----
JM-WE5-1	-----	-----	-----	-----	-----	-----	-----R	-----	-----	-----
JM-WE5-2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE6-1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE6-3	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE6-4	-----	-----	-----	-----	-----	-----D	-----D	-----S	-----	-----
JM-WE7-2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE7-6	-----Y-H-R	-----P	-----R	-----S	-----	-----	-----	-----	-----	-----
JM-WE7-9	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE8-1	C	-----C	-----APP	-----V-R	-----D	-----S	-----E	-----*	-----D	-----D
JM-WE8-2	-----L-R	-----R-S	-----R	-----S	-----E	-----W-D	-----D	-----S	-----E	-----R
JM-WE8-3	-----L-R	-----R-S	-----R	-----I	-----S	-----E	-----W-D	-----D	-----S	-----E
JM-WE8-4	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE8-5	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE8-8	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE9-1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE9-2	-----	-----	-----	-----S	-----E	-----D	-----D	-----S	-----*	-----
JM-WE9-4	-----	-----	-----	-----	-----	-----D	-----D	-----S	-----	-----
JM-WE9-7	-----Y-H-R	-----P	-----R	-----S	-----	-----	-----	-----S	-----	-----
JM-WE9-8	-----	-----	-----	-----	-----	-----VD	-----D	-----S	-----	-----
JM-WE9-9	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE10-1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE10-3	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JM-WE10-6	-----	-----	-----	-----	-----	-----D	-----D	-----S	-----	-----

Fig. 2. Amino acid sequence alignments of the HERV-W env fragments. Consensus sequences are shown on the top row. Dashes indicate residues identical to the consensus sequences and dot indicates gap introduced to maximize the alignments. Frameshifts are indicated by a slash and stop codons are shown in an asterisk.

CONSENSUS	SKPEAQLWSQ	QVKYLGLKLS	KGTRALSEE-	IQPILAYPNP	KTLKELRGLL	GITGFCRIWI	PRYGRIARPL	YTLI/ETQKA	NTHLVRWTP	AEVAFQALKK	ALTQAPVLNL	110
HERV-Wpo1	-K-R-C-	E-R			H-	Q-F	KQ	TP	R	Y	T	FS
JM-WP2-2												
JM-WP2-4												
JM-WP2-10	-K-C	S		H	Q	Q-SF	SE	KK	V	S	K	S
JM-WP2-12	-K-C	N-V		H	Q	Q-F	SE	K	A	Y	AA	V
JM-WP3-2												
JM-WP3-11												
JM-WP6-1	-T-C			H	Q	F	T	DG	E	K	R	IL
JM-WP6-2	-K-C			LTH	A	Q-F		C	K	K	E	/-A
JM-WP6-3												
JM-WP6-5												
JM-WP6-6												
JM-WP6-7	-K-C	L		H	Q	S	/I	Q	E	K	R	IL
JM-WP6-9	-K-C			H	Q	S	F	T	DG	E	K	R
JM-WP7-2	-K-C	P	G	H	Q	S	/I	Q	E	K	R	IL
JM-WP7-3	P-K-C	L		H	Q	S	/I	Q	E	K	R	IL
JM-WP7-4	-K-C	P		H	Q	S	/I	Q	E	K	R	IL
JM-WP7-5	-K-C	L		H	Q	S	/I	Q	E	K	R	IL
JM-WP7-6												
JM-WP7-7	-K-C	L		H	Q	S	/I	Q	E	K	R	IL
JM-WP7-8	P-K-C	PE		H	Q	S	/I	Q	E	K	R	IL
JM-WP7-9												
JM-WP8-1												
JM-WP8-2												
JM-WP8-5	-K-C	P		H	Q	S	/I	Q	E	K	R	IL
JM-WP8-9												
JM-WP9-1												
JM-WP9-4												
JM-WP9-7												
JM-WP10-1												
JM-WP10-2												
JM-WP10-3												

CONSENSUS	PAGQDFSLYV	TEKTGIALGV	LTQIRGMSLQ	PV---AYLS	KEIDVVAKGW	PHCLQVVAV	AVLVSEAVKI	IQGRDLTVWT	AHDVNSILTA	KRDLWLSDNH	LSLKYQALLL	EGP
HERV-Wpo1	-T-----A		S	V		W	M		S	G	G	N
JM-WP2-2			*					*	V		H	X
JM-WP2-4												RA
JM-WP2-10	-TR / -N	P	H	I	E*SAG/	R	T	T	A	S	G	R
JM-WP2-12	SM		TR	P		R	V		G	S	G	A
JM-WP3-2			*					*	V	R		H
JM-WP3-11								*	V	R		
JM-WP6-1	-T	AL	T	S	Q	R		I	S	G	G	L
JM-WP6-2	-MEE	H	T				A		*	V	R	
JM-WP6-3			*					*	V	R		
JM-WP6-5			*					*	V	R		
JM-WP6-6			*					*	V	R		
JM-WP6-7	-T	V	I	M	KT	R		F	Y	S	G	P
JM-WP6-9	-T	AL	T	S	Q	R		I	SR	G	G	L
JM-WP7-2	-T	V	T	M	KT	R		F	Y	S	G	R
JM-WP7-3	-T	V	T	M	KT	R		F	Y	S	G	R
JM-WP7-4	-T	V	T	M	KT	R		F	Y	S	G	R
JM-WP7-5	-T	V	I	M	KT	R		F	Y	S	G	R
JM-WP7-6			*					*	V	R		
JM-WP7-7	-T	V	I	M	KT	R		F	Y	S	G	R
JM-WP7-8	-T	V	I	M	KT	R		F	Y	S	G	R
JM-WP7-9			*					*	V	R		
JM-WP8-1			*					*	V	R		
JM-WP8-2		A	*					*	V	R		
JM-WP8-5			*					*	V	R		
JM-WP8-8	-T	V	I	M	KT	R		F	Y	S	G	R
JM-WP9-1			*					*	V	R		
JM-WP9-4			*					*	V	R		
JM-WP9-7		A	*					*	V	R		
JM-WP10-1		A	*					*	V	R		
JM-WP10-2		A	*					*	V	R		
JM-WP10-3		A	*					*	V	R		

Fig. 3. Amino acid sequence alignments of the HERV-W *pol* fragments. Consensus sequences are shown on the top row. Dashes indicate residues identical to the consensus sequences and dot indicates gap introduced to maximize the alignments. Frameshifts are indicated by a slash and stop codons are shown in an asterisk.

been growing interest in the possible biological roles of endogenous retroviral sequences, in particular, regulation of HERV expression. Tissue-specific expression of HERV-R and HERV-K was found in developing human tissues (Andersson *et al.*, 2002) and expression potentially immunosuppressive protein-coding HERV- H *env* transcripts has been detected in various normal and malignant cell types (Mangeney *et al.*, 2001). With structural and Northern analysis, active HERV-E gene was identified in human pancreas and thyroid tissues, suggesting that they may play a role in physiological function to common to these gland tissues (Shiroma *et al.*, 2001). In addition, HERV-E *env* gene was actively expressed in prostate carcinoma tissues and cell lines, but not in normal prostate tissues and cells using RT-PCR, RNA *in situ* hybridization and Northern blot analyses (Wang-Johanning *et al.*, 2003). HERV-K *env* transcripts have been detected in most breast cancer cell lines and many breast tumor tissues (Wang-Johanning *et al.*, 2001). Those expressions might be relevant to various occasions such as hormone producing status (thyroid and adrenal gland etc.) and immune function, and to specific sequences

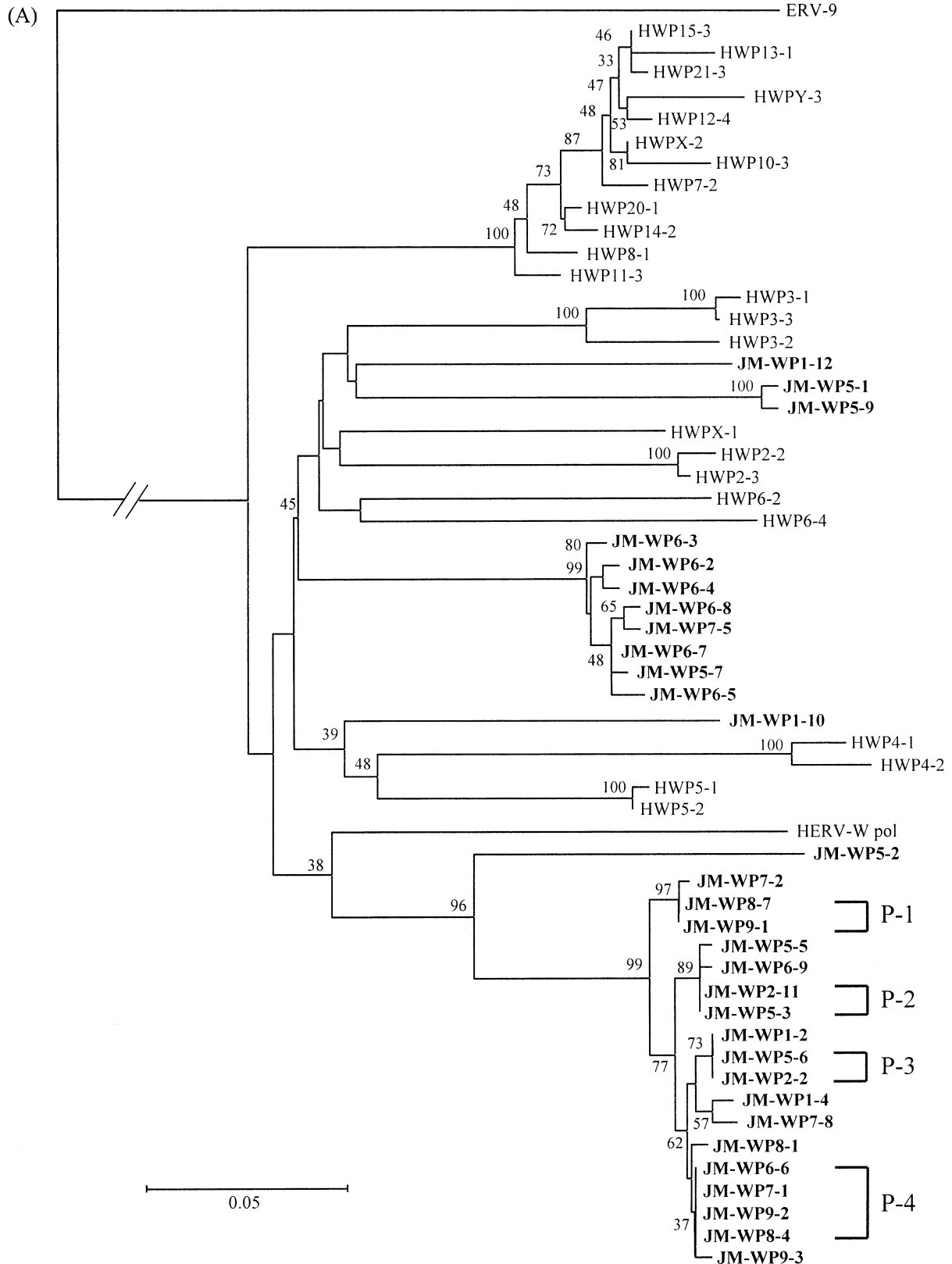
in the HERV LTR or in the vicinity of the proviral insertion sites. More strongly, it has been supported that enhancer and promoter elements in retroviral LTRs can influence the transcription of neighboring genes (Kowalski *et al.*, 1999). This can result in transcriptional activation or gene silencing and in changes in tissue specificity of expression (Schulte *et al.*, 1996; Ting *et al.*, 1992). Moreover, two HERV-E LTRs, which act as alternative promoters for cellular genes, have been identified using human genome sequence database screening (Kowalski *et al.*, 1999). Recently, it has been reported that HERV-E LTRs contribute to the expression of the human apolipoprotein C1 (APOC1) and endothelin B receptor (EDNRB) genes by providing alternative promoters (Landry and Mager, 2003).

In the present study, we identified HERV-W elements that are expressed in normal monkey tissues with RT-PCR approach and sequence analysis. The identical sequences were found in different tissues of the monkey, indicating that they could have biological roles with neighboring genes in monkey tissues. This information will allow us to explore the potential transcriptional role of HERV-W in the monkey and

human tissues for further studies.

The relationship of HERV elements and diseases has been discussed constantly over the past few years. The

expression of HERVs associated with a number of chronic human disease including multiple sclerosis, diabetes, and autoimmune arthritis (Perron *et al.*, 1997; Conrad *et al.*,



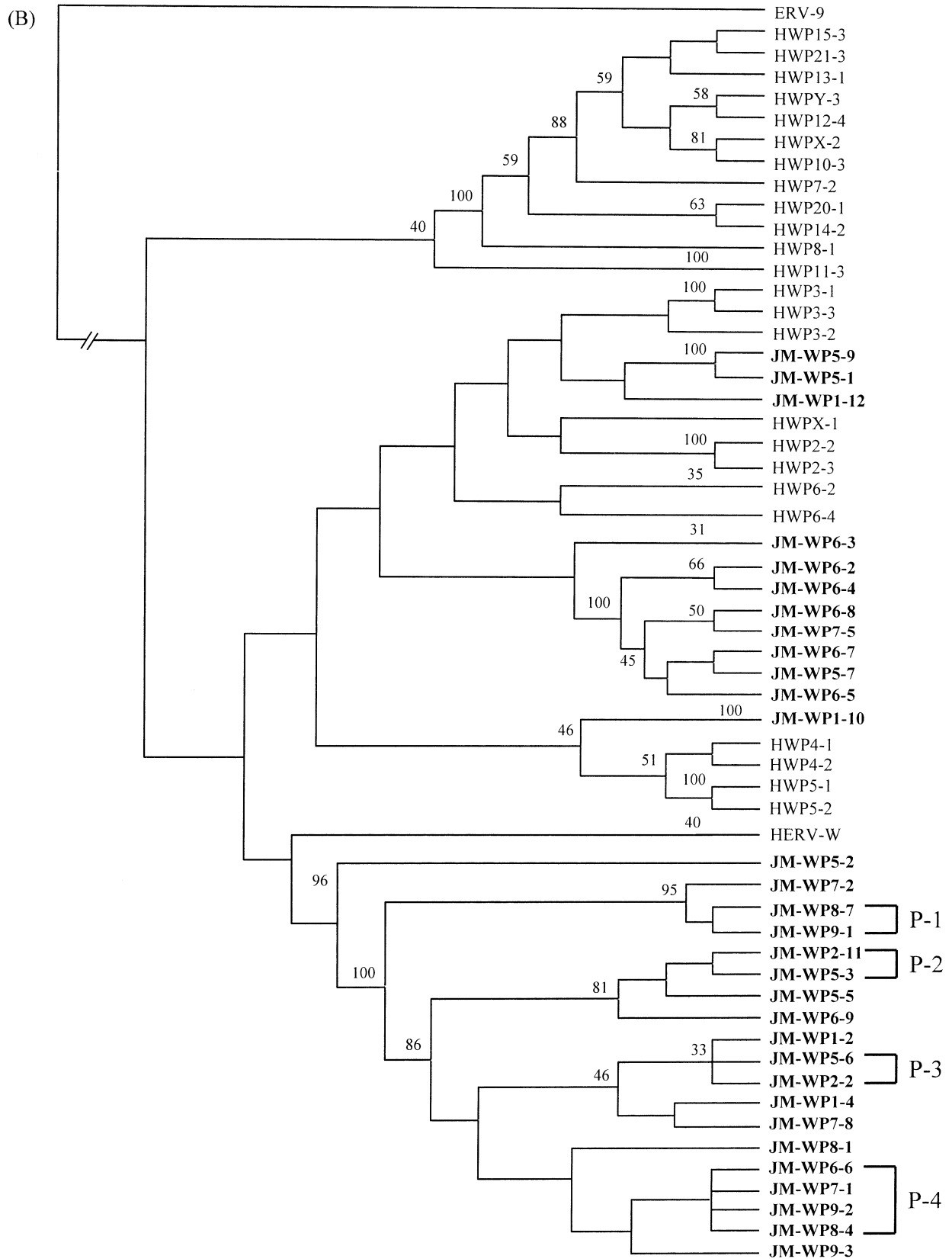
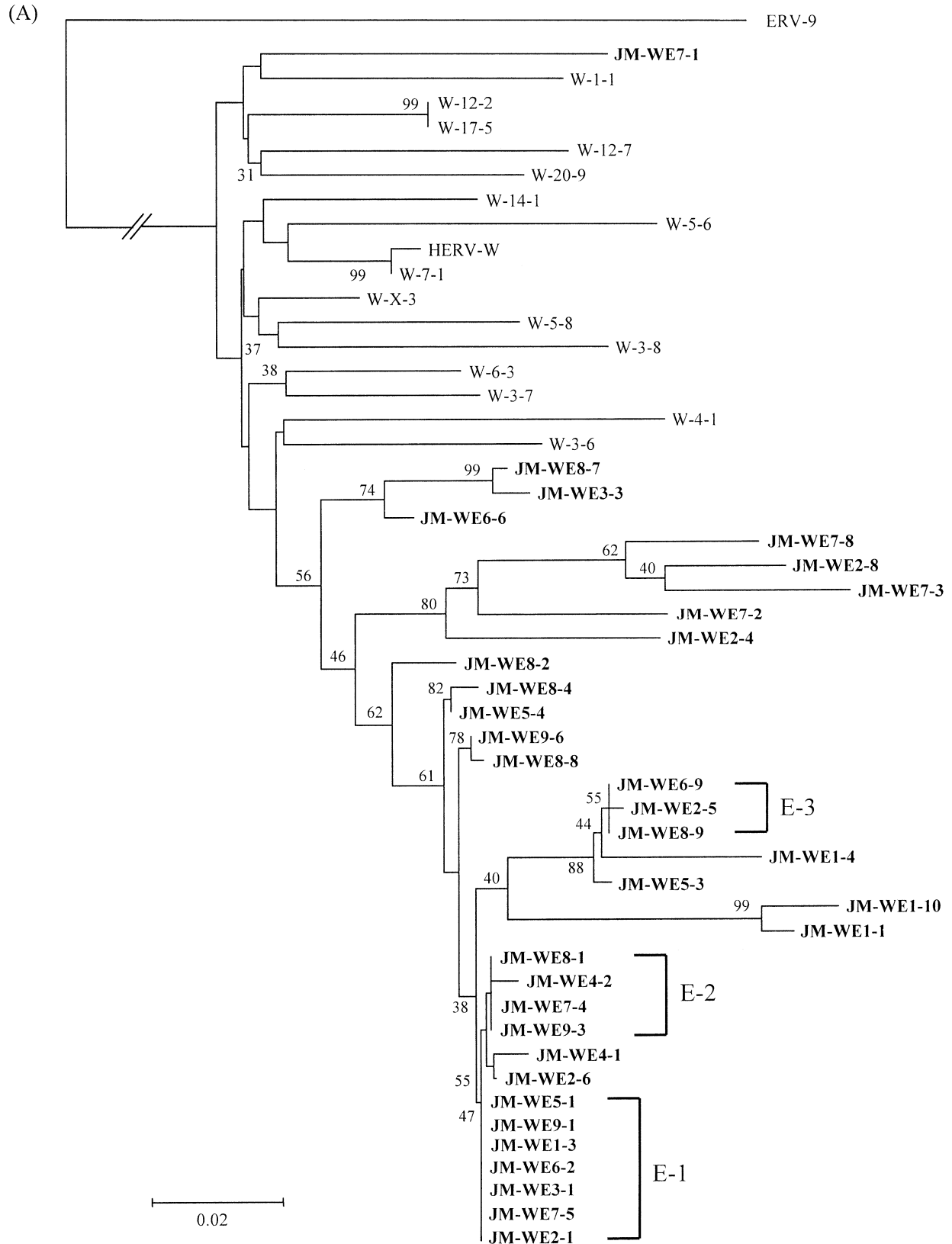


Fig. 4. Phylogenetic tree for *pol* gene sequences of HERV-W in human and Japanese monkey obtained by the neighbor-joining (A) and maximum parsimony methods (B). Identical sequences of HERV-W *pol* fragments from different monkey tissues were described as P-1, P-2, P-3, and P-4. Branch lengths are proportional to the distances between the taxa. The values at the branch points indicate the percentage support for a particular node after 100 bootstrap replicates were performed. ERV-9 was used as an outgroup.

1997; Nakagawa *et al.*, 1997) is only speculative. HERVs have frequently been proposed as etiological cofactors in chronic diseases such as cancer, autoimmunity and neuro-

logical disease (Lower, 1999). Recently, retroviral sequences belonging to HERV-W family were identified in brain of individual with schizophrenia, increasing the possibility



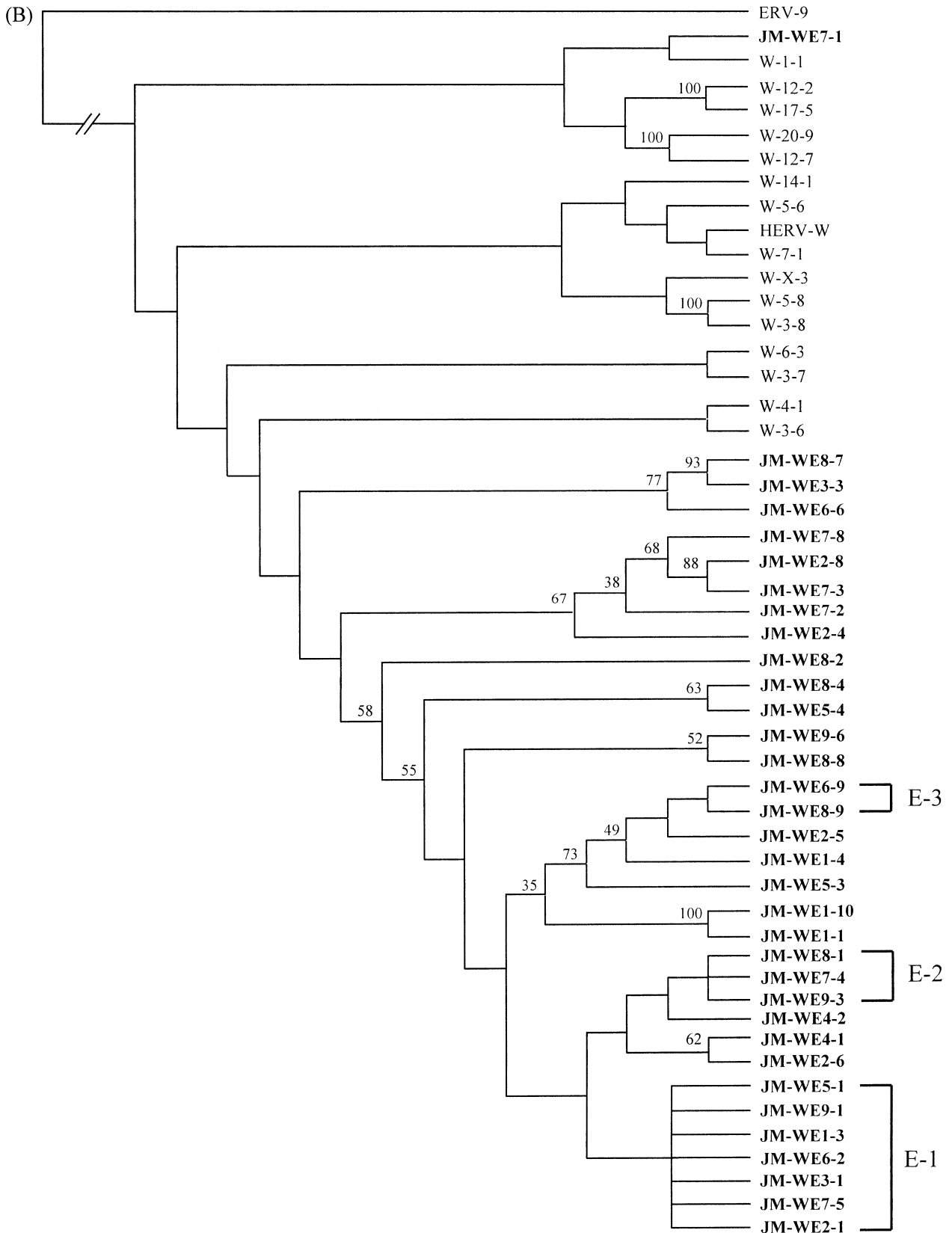


Fig. 5. Phylogenetic tree for *env* gene sequences of HERV-W in human and Japanese monkey obtained by the neighbor-joining (A) and maximum parsimony methods (B). Identical sequences of HERV-W *env* fragments from different monkey tissues were described as E-1, E-2, and E-3. Branch lengths are proportional to the distances between the taxa. The values at the branch points indicate the percentage support for a particular node after 100 bootstrap replicates were performed. ERV-9 was used as an outgroup.

that HERV-W family could be involved in human brain disease (Karlsson *et al.*, 2001). To examine the role of HERV in human cancer, we have previously reported that HERV-W *env* gene sequences had been characterized in some human cancer cell lines (Yi *et al.*, 2002). This report adds to the pathological knowledge of HERV family about diverse human disease including cancer. Here, identification of HERV-W gene sequences from various monkey tissues is first step to approach of understanding their biological role in monkey tissues. For further studies, comparative analyses between humans and monkeys are necessary in order to improve knowledge about the evolutionary mechanism of HERV elements during primate evolution and their particular pathogenic role in various tissues related to diseases.

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