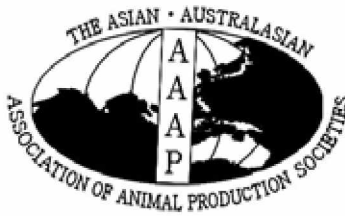

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Insertional Variations of Two Porcine Endogenous Retroviruses (PERVs) in Korean Native Pigs and Asian Wild Boars

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ABSTRACT : Porcine Endogenous Retroviruses (PERVs) are a major concern in relation to xenotransplantation. Previous research indicated that PERVs are present at about 50 copies in the pig genome and their chromosomal insertion sites are different among pig breeds. We examined nine Korean native pigs and seven Asian Wild Boars for the presence of a PERV-A at SSC 1q2.4 and a PERV-B at SSC 7p1.1-2 previously reported in a Large White pig. The PERV-B at locus 7p1.1-2 displayed insertional variability in Korean native pigs and Asian Wild Boars. Using the primers for the PERV-A at 1q2.4 from Large White pig, we only can amplify an unclassified 798 bp sequence, which showed insertional variability only in Korean native pigs. This study indicates that there are differences within and between Asian and European pigs in PERV insertions and suggests that selection could generate PERV-free lines of pigs more suitable for xenotransplantation. (**Key Words** : Pigs, Insertional Variations, Porcine Endogenous Retroviruses (PERVs), Xenotransplantation)

INTRODUCTION

The pig (*Sus Scrofa*) is regarded as the best xenotransplant organ donor for humans for various reasons and pre-clinical trials already have been carried out with pig xenografts (Chari et al., 1994; Groth et al., 1994; Deacon et al., 1997; Lee and Moran, 2001). Since the first description of PERV (Porcine Endogenous Retrovirus), little attention has been paid to this type-C virus until Patience et al. (1997) reported that a porcine kidney cell line released PERV particles capable of infecting human cells. The *in vitro* porcine retroviral infection of human cells was confirmed by other research groups (Martin et al., 1998a; Patience et al., 1998; Wilson et al., 1998; Deng et al., 2000; van der Laan et al., 2000) but there has been no evidence of *in vivo* infection in baboons (Martin et al., 1998b) or in humans (Paradis et al., 1999; Pitkin and Mullon, 1999; Switzer et al., 1999).

Porcine gamma retroviruses are classified as PERV-A, PERV-B and PERV-C, based on their envelope proteins. They are present at approximately 30 to 50 copies in the porcine genome (Le Tissier et al., 1997; Akiyoshi et al., 1998) and the PERV integration sites vary between breeds (Lee et al., 2002; Gorbovitskaia et al., 2003). Rogel-Gaillard et al. (1999; 2001) reported 21 proviral PERV loci by screening a Large White genomic BAC (Bacterial Artificial Chromosome) library. Two sites, a replication competent PERV-A at SSC 1q2.4 (from BAC clone 130A12) and a replication non-competent PERV-B at SSC1.1-2 (from BAC clone 484G4), have been further investigated in this study in Korean native pigs and Asian Wild Boars.

MATERIALS AND METHODS

Animals

Nine Korean native pigs and seven Asian Wild Boars were used in this study. Although taken to the brink of extinction by the inflow of European exotic genes into the

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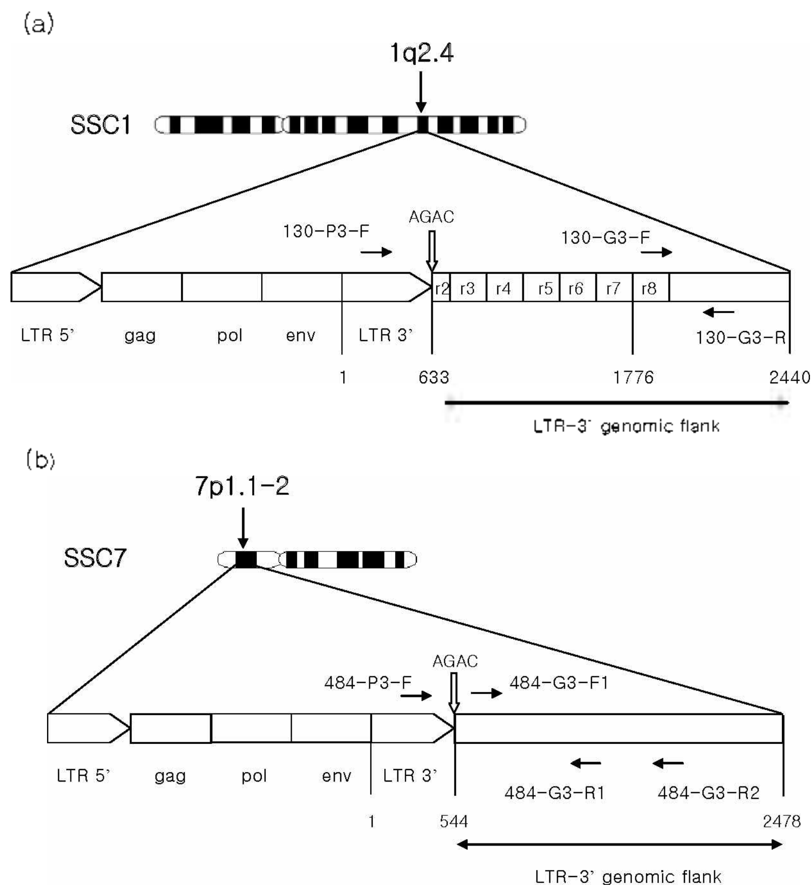

Figure 1. Schematic representation of the two PERV genomic integration loci used in this study. Genomic organizations of the PERV-A locus at 1q2.4 (a) and PERV-B locus at 7p1.1-2 (b). The primer positions are also indicated. This figure is modified from Gorbovitskaia et al. (2003).

Korean pig populations beginning about 50 years ago, an intensive recovery program, commenced a decade ago mainly by the National Livestock Research Institute in Korea and using pigs that were likely native on the basis of morphological characteristics, has rescued the Korean native population. Nine unrelated Korean native pigs were chosen based on known pedigree information. Asian wild boar samples were collected from different private wild pig farms located at different geographical regions in Korea. These boars were also very unlikely to be related to each other.

Oligonucleotide primers

Four primer pairs from genomic flanks and PERV-genomic junctions were used for screening for the presence/absence of the target PERVs (Gorbovitskaia et al., 2003). Primer 130-P3-F from the viral LTR (Long Terminal Repeat) in conjunction with primer 130-G3-R tests for the presence of the PERV-A locus at 1q.24. Primer 130-G3-F and 130-G3-R allow positive control amplification from the 3' flank of this PERV-A site. Similarly primer 484-P3-F from the viral LTR of the PERV-B at 7p1.1-2 (Gorbovitskaia et al., 2003) in conjunction with primer 484-G3-R2 tests for the presence of this viral insert whereas 484-G3-F1 and 484-G3-R1 allow positive control amplifications from the genomic flanking sequence. The primer locations are illustrated in Figure 1.

Polymerase chain reaction (PCR), cloning and sequencing

Polymerase chain reaction was performed in 25 μl volumes, each containing 50 ng of template DNAs, 40 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.4 pmol of each primer, 1.6 mM of each dNTP and 2.5 unit of *Taq* polymerase (Bioneer, Korea). Reaction profiles included a 5 min denaturation step at 94°C followed by 40 cycles, each consisting of 30 sec denaturation at 94°C, 30 sec annealing at optimum temperature (see Gorbovitskaia et al., 2003), 1 min of extension at 72°C, and then a final 10 min extension step at 72°C using a GeneAmp® PCR system 2700 (Applied Biosystems, Inc., USA). The PCR products were separated by electrophoresis on a 1.5% agarose gel and cloned into the pGEM®-T Easy plasmid vector (Promega, USA) for sequencing.

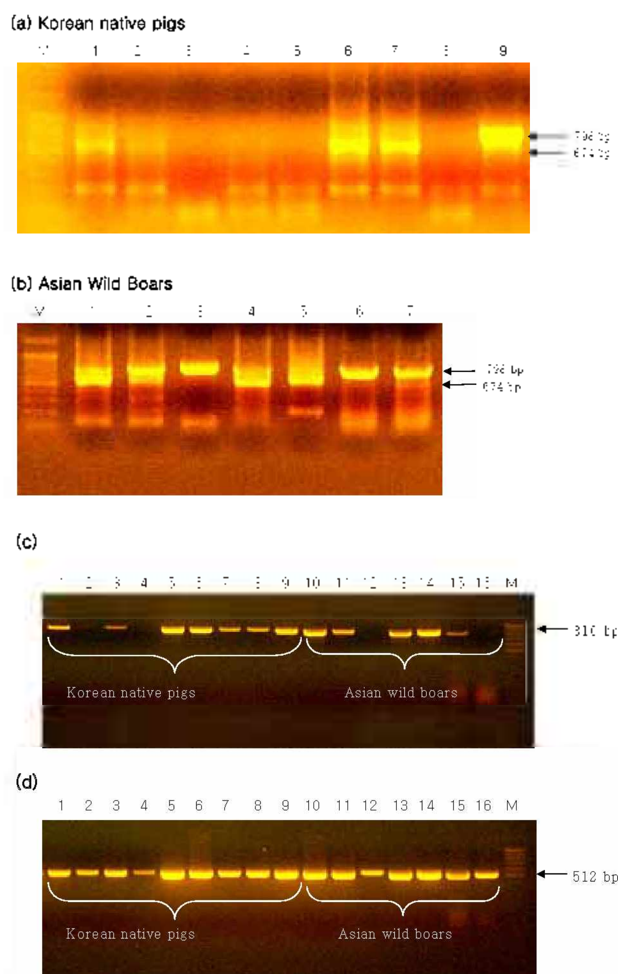

Figure 2. PCR test for pig variability for the presence/absence of unmapped PERV-A from nine Korean native pigs (a) and from seven Asian Wild Boars (b) using the primers designed from locus 1q2.4 of Large White. Two PCR products, 798 and 674 bp, were observed and neither of the products are the expected PCR products of 1,844 bp. PCR Amplication of the PERV-B 3' junction (c) and genomic flank (d) at the 7p1.1-2 locus of Korean native pigs and Asian Wild Boars. Lane M: 100 bp size standards, Lanes from 1 to 9: Korean native pigs, Lanes from 10 to 16: Asian Wild Boars.

RESULTS AND DISCUSSION

Amplification by PCR using the PERV LTR-primers in conjunction with the relevant genomic junction primers indicated the presence of PERV-B at 7p1.1-2 with the expected product size in most of the Asian pigs. However the PCR amplifications for PERV-A at 1q2.4 gave smaller than expected PCR products. The positive control amplicons from the 3' genomic flanks (Gorbovitskaia et al., 2003) were produced from all the animals indicating that all template DNAs were of adequate quality (data not shown).

For the putative 1q2.4 site, primers 130-P3-F and 130-G3-R amplified a product of 798 bp from one Korean native pig, four others had a weaker amplicon of 674 bp and four animals had no PCR product with these primers (Figure 2a). All the Asian Wild Boars gave PCR products of 798 bp with these primers, with the 674 bp bands also appearing in four animals (Figure 2b). However neither amplicon matches the expected PCR product size of 1,839 bp for the 1q2.4 PERV-A locus reported by Gorbovitskaia et al. (2003). Consequently, the 798 bp and 674 bp PCR products were sequenced to determine whether they matched the 1q2.4 PERV-A and flanking genomic sequences. These sequences indicated that the 798 bp PCR fragment matches the porcine BAC clone 130A12 sequences with expectation value of 0.0, with the discrepancy in size being due to the occurrence of seven 190 bp repeats after the 3' end of PERV LTR in the 130A12 clone sequence, while the Korean native pig had only one 103 bp degenerate repeat. The 674 bp PCR product had no homology with the PERV 3' LTR sequences, even though the genomic flank sequences are well matched with the 130A12 sequences. Subsequently, new primers were designed for radiation hybrid (RH) mapping, developed by Yerle et al. (1998), from the genomic flank sequences conserved between the 798 bp and 1,839 bp products, to confirm whether they came from the same locus at 1q2.4 reported by Gorbovitskaia et al. (2003). The RH typing results showed that all the panels were positive, indicating the presence of this sequence at multiple locations in the porcine genome (data not shown). Subsequently, the genomic flank sequences were analysed with the RepeatMasker program (http://www.repeatmasker.org/) to determine whether the genomic flank contains repetitive elements. The results indicated that the genomic flanking sequence was actually porcine LINE (Long Interspersed Nuclear Element) L1 family. Thus it is impossible to determine with certainty whether PERV-A is present or absent from 1q2.4 in the screened Korean native pigs and Asian Wild Boars. However, Gorbovitskaia et al. (2003) indicated that there an additional band was observed when using the primers from 1q2.4. Therefore it is more likely that the 798 bp PCR product was from another locus. If there is an insertion at 1q2.4 in the Asian pigs, the different sized PCR product found in these pigs when compared to that from the Large White implies that it is different from what was detected by Gorbovitskaia et al. (2003).

For the 7p1.1-2 site, primers 484-P3-F and 484-G3-R2 amplified products of 810 bp for seven out of nine Korean native pigs and five out of seven Asian Wild Boars (Figure 2c). All the DNA samples amplified the positive control amplicon of 512 bp using primers 484-G3-F1 and 484G3-R1 from the 3'genomic flank (Figure 2d). Both PCR products were exactly of the sizes found by Gorbovitskaia et al. (2003). Thus some Korean native pigs and Asian Wild Boars have the PERV-B insert at locus 7p1.1-2, but other

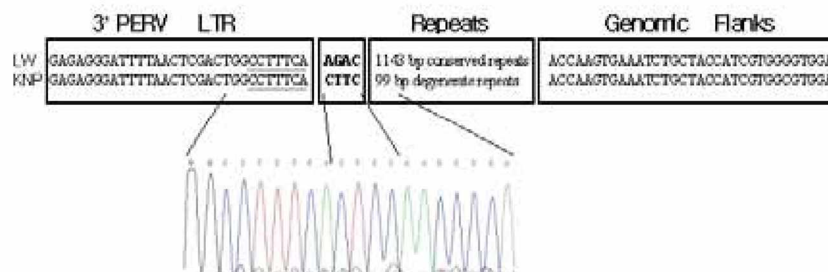

Figure 3. Sequence alignment of partial segments of the junction at the 3' PERV LTR and genomic flank for the locus 1q2.4 in a Large White (LW) from BAC clone 130A12 and a Korean native pig (KNP). Further experiments indicated that the KNP sequence used here came from an unknown locus. The four genomic nucleotides found at the integration locus are in bold letters and the seven bp viral motif is underlined. The electrophoregram of the 3' PERV junction in a Korean native pig is also presented.

animals lack it. Furthermore it is tempting to speculate that either some animals are heterozygous for the presence/absence of this insert or the primer sequences have polymorphisms. For example, Korean native animal 3 and Asian Wild Boar 15 have distinctly weaker amplification of the junction fragment, compared with Korean native animals 5 and 6, but strong amplification of the control flanking fragment (Figure 2c and 2d).

The sequence of the unmapped 798 bp PERV amplicon (Figure 3) from the Korean native pig indicated that the sequence had the expected seven conserved nucleotides (CCTTTCA) at the end of the 3' LTR. This implies that the 798 bp PERV amplicon comes from PERV-A since PERV-B has a different seven conserved nucleotides, TCTTTCA (Czauderna et al., 2000; Niebert et al., 2002; Gorbovitskaia et al., 2003). Recently a porcine BAC library was constructed from a Korean native pig boar (Jeon et al., 2003) and investigation of the PERV inserts from this library will give a better idea of PERV integration sites and their flanking sequences in Asian pigs.

In conclusion, this study showed insertional variability of PERV-B at 7p1.1-2 in Korean native pigs and Asian Wild Boars. Meanwhile an unmapped 798 bp PERV amplicon produced using primers from Large White PERV-A at 1q2.4 indicates that the insertional variability is only observed in Korean native pigs. For the successful xenotransplantation, pigs free of replication competent PERV would be very valuable. These results suggest that if enough breeds are studied it may be possible to breed a PERV-free line of pigs.

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