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**Phylogenic analysis of the SLA-1 gene variants observed from
Korean Jeju pig** (Brief Report)

(Phylogenetische Analyse von SLA-1 Genvarianten beim koreanischen Jeju Schwein)

Background: The Swine Leukocyte Antigen (SLA) class I consists of SLA-1, SLA-2, and SLA-3 genes (CHARON et al., 2000) that are expressed on the surface of most nucleated cells. Pig organs mainly from miniature pigs have been used in studies on xenotransplantation (SMITH et al., 2005). It is important to understand genetic variations of the SLA-1 gene considering the SLA-mediated immune responses. Collecting information on single nucleotide polymorphisms (SNPs) and alleles of SLA-1 and their phylogenic relationships is important regarding future uses of the Korean Jeju Pig (KJP) in xenotransplantation.

Procedure

Primer sequences:

Primers were designed from the swine coding sequence (GenBank accession No.: AY247766 and AF464016) of porcine SLA-1 consisting of 8 exons in 1,087 bp length.

forward primer: 5'-GGGCGTCGACCAGACTCCGAGGCTGAGG-3' (nt1-28)

reverse primer: 5'-GAAGGTTCTCAATCCTTCCATTT-3' (nt1,544-1,566)

Identification of SLA-1 alleles:

Spleen tissues were collected from 23 individuals of the breed Korean Jeju Pig at an age of 30 weeks at National Institute of Animal Science, and total RNA was extracted. First strand cDNA was synthesized from pooled spleen tissues by random primers with Reverse Transcriptase. To amplify cDNAs SLA-1 specific primers were used with 2.5 µl of 10 X reaction buffer, 10 µM dNTP, 1 µl of each primer (10 pM), 50 ng of cDNA, and 2 units of *Taq* DNA polymerase in a final volume of 25 µl. After heating at 95°C for 2 min, a total of 35 cycles was adapted for denaturation at 94°C / 1 min, annealing at 57°C / 1 min, and polymerization at 72°C / 2 min. The PCR products were inserted into pGEM T easy vector for blue and white selection with DH10B competent cells. The selected clones were sequenced.

Results: A total of 120 clones sized approximate 1,500 bp were produced. Finally, 46 clones were clustered with the Seqman program of DNASTAR version 6.1 revealing 35 SNPs. Sequences of polymorphic KJP clones were submitted to GenBank (GenBank accession No.: DQ992486-92). The alignments contained several SNP positions, and especially, there were several nucleotides at positions 165 (A/C/T), 166 (A/C/T), and 251 (G/C/A) based on the DQ992492 representing a consensus sequence of KJP. A phylogenic tree was constructed with DNADIST option (DNA distance matrix, BioEdit version 7.0.1) for identifying a neighbor-joining phylogenic relationship. The identified consensus sequences (DQ992492) were submitted to the MHC Immuno Polymorphism Database (IPD) of the Major histocompatibility complex (MHC) with a

new accession allele number SLA-1*w12hy01. The complete cDNA sequences of SLA-1 allele representing major alleles from NCBI have been aligned with the consensus sequences of KJP. As shown in the Figure, the KJP was closely clustered with the SLA-1*w12-w12Lw01 allele (Large White breed). This study provides new information on the genetic characteristics of KJP and phylogenic MHC diversity.

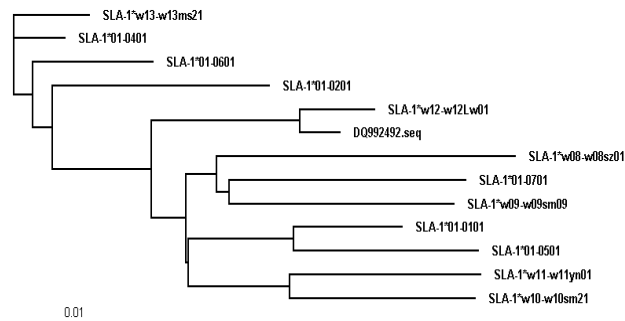


Figure: Phylogentic relationship among 13 SLA-1 alleles (Phylogenetische Beziehung von 13 SLA-1 Allelen) The GenBank accession No. of the SLA-1 alleles: AF464045 (SLA-1*01 0101), AF100665 (SLA-1*01 0201), AF014005 (SLA-1*01 0401), AF464044 (SLA-1*01 0501), AY135593 (SLA-1*01 0601), AF464036 (SLA-1*01 0701), AF464013 (SLA-1*01 w08sz01), AY135594 (SLA-1*01 w09sm09), AY135589 (SLA-1*01 w10sm21), AY102469 (SLA-1*01 w11yn01), AY247766 (SLA-1*01 w12Lw01), AY459297(SLA-1*01 w13ms21).

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