

Sequence-based characterization of the eight SLA loci in Korean native pigs

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Summary

Eight swine leucocyte antigen (SLA) gene (SLA-1, SLA-2, SLA-3, SLA-6, DRA, DRB1, DQA, DQB1) alleles were identified using sequence-based typing method in three Korean native pigs used for breeding at the National Institute of Animal Science in Korea. Six new alleles in class I genes and three new alleles in class II genes have been identified in this breed and can give valuable information for xenotransplantation and disease resistance.

The major histocompatibility complex (MHC) antigens in pig are called the swine leucocyte antigens (SLA) and they can be divided into class I (SLA-1, -2, -3) and class II (SLA-DR, -DQ) antigens based on their structure and function (Smith *et al.*, 2005a,b). These SLA genes are highly polymorphic and play very important roles in the immune response to infectious diseases. Many studies have associated SLA types with disease resistance and various economic traits (Gautschi & Gaillard, 1990; Mallard *et al.*, 1991). Recent studies have also focused on using pigs as a possible source of organ grafts for human, which makes the study of SLA antigens important for overcoming this potential immunological barrier in xenotransplantation (Xu *et al.*, 1999; Ramachandran *et al.*, 2004). We are interested in developing a resource herd of SLA-defined pigs for biomedical (and agriculture) research as well as a potential source of xenograft donors using the Korean native pigs. In this study, we have characterized three SLA class Ia genes (SLA-1, SLA-2, SLA-3), one SLA class Ib gene (SLA-6) and four SLA class II genes (DRA, DRB1, DQA, DQB1) in three Korean native pig boars, which have been used for breeding at the National Institute of Animal Science in Korea.

Sequence-based typing (SBT) was performed by reverse transcription-polymerase chain reaction (RT-PCR) followed by cloning and sequencing of multiple clones for each SLA gene as described previously (Lee *et al.*, 2005). Briefly, RNA was extracted from each blood sample using Trizol reagent (Gibco/BRL, Gaithersburg, MD, USA) according to the manufacturer's instructions. The extracted RNA was denatured at 70 °C for 10 min, and reverse transcription was performed in reactions containing 5 mM MgCl₂, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% TritonX-100, 1 mM of each dNTP, 0.5 U of recombinant RNasin ribonuclease inhibitor, 15 U of AMV reverse transcriptase (Promega, Madison, WI, USA) and 500 ng oligo (dT) primer at 42 °C for 1 h. Using this cDNA as a template, locus-specific PCRs were performed using Amplitaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) and locus-specific primers (Lee *et al.*, 2005). Thermal cycling was performed in a GeneAmp PCR system 2700 (Applied Biosystems). Reaction profiles included a 10-min denaturation step at 94 °C followed by 38 cycles, each consisting of 30 s of denaturation at 94 °C, 30 s of appropriate annealing temperature between 60 and 64 °C, 1 min of extension at 72 °C, and then a final 10 min extension step at 72 °C. Cloning was carried out using pGEM-T Easy plasmid vector (Promega) and multiple clones were sequenced. For each SLA gene, more than three clones were sequenced and analysed. A second RT-PCR and cloning reaction was performed for each SLA gene to confirm the polymorphic sequences. New SLA alleles were subjected to allele name assignment by the International Society for Animal Genetics (ISAG) SLA Nomenclature Committee based on a combination of phylogeny and sequence comparison (Smith *et al.*, 2005a,b).

A total of 23 alleles were identified at eight SLA loci in the three Korean native pig boars (Table 1). Their nucleotide and amino acid sequences have been deduced and submitted to the GenBank database. The detailed information of these alleles, including the phylogenetic analyses with all other known SLA alleles and multiple sequence alignments, is available at the SLA section of the Immuno Polymorphism Database (IPD-MHC) website (<http://www.ebi.ac.uk/ipd/mhc/sla/>) (Ellis *et al.*, 2006). Comparison with previously identified SLA alleles indicated that nine of them were novel and therefore assigned tentative allele names by the ISAG SLA Nomenclature Committee. In addition, five alleles were found to be

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Table 1. The swine leucocyte antigen (SLA) class I and class II alleles identified in three Korean native pigs

Locus/Pig ID	1205	1159	1119
SLA-1	w11jh01 ^a 0801 ^b	w11jh01 ^a	w11jh02 ^a
SLA-3	0303 0701	0303	05jh01 ^a
SLA-2	jh01 ^a 0502 ^b	jh01 ^a	jh02 ^a
SLA-6	w04jh01 ^a 0101	w04jh01 ^a	0102
DRA	020202 ^b 0201jh01 ^a	020202	010101
DRB1	1101 ^b 10jh01 ^a	1101	0101
DQA	02jh01 ^a 0101	02jh01 ^a	0101
DQB1	0503 ^b 0601	0503	0101

^a New alleles; ^b Confirmed alleles.

The nucleotide sequence data reported here have been submitted to the DDBJ/EMBL/GenBank nucleotide databases under the accession numbers DQ883208–DQ883227 and EF589959–EF589961.

identical to the tentative alleles previously recognized (SLA-1*w08sz01, SLA-2*05sz01, SLA-DRA*0202 mm16, SLA-DRB1*w11an01 and SLA-DQB1*05an01) (Smith *et al.*, 2005a,b). These alleles were therefore renamed as confirmed alleles SLA-1*0801, SLA-2*0502, SLA-DRA*020202, SLA-DRB1*1101 and SLA-DQB1*0503, respectively. Furthermore, results suggested that boar 1159 was homozygous for a novel SLA haplotype (Hp-56.30) while boar 1119 was homozygous for another novel class I haplotype and the Hp-0.1 class II haplotype which was identical to the H01 haplotype found in the Large White pig breed (Smith *et al.*, 2005b). Therefore, the assigned SLA haplotype of animal 1119 is Hp-59.1. This suggested some common genetic background between the European breeds and the Korean native pigs. In order to improve the production ability of native pigs in Korea, it was also possible to crossbreed with the highly productive pigs such as Large White. Comparison of the alleles also suggested that boar 1205 was heterozygous for SLA haplotypes Hp-7.23 and Hp-56.30 (Smith *et al.*, 2005a). Typing of the offspring is necessary in the future to show the inheritance and segregation of the alleles as haplotypes and therefore provide further confirmation for haplotype assignments in these three pigs. In conclusion,

these new SLA alleles and haplotypes contribute to the understanding of polymorphism of these genes and give us better tools to study the role of SLA antigens in xenotransplantation and in important agricultural traits such as disease resistance.

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