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Molecules and Cells

Establishment of a Resource Population of SLA Haplotype-Defined Korean Native Pigs

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The highly polymorphic porcine major histocompatibility complex (MHC), or the swine leukocyte antigens (SLA), has been repeatedly associated with variations in swine immune response to pathogens and vaccines as well as with production traits. The SLA antigens are also important targets for immunological recognition of foreign tissue grafts. We recently established a resource population of Korean native pigs as models for human transplantation and xenotransplantation research. In this study, 115 animals derived from three generations of the Korean native pigs were genotyped for three SLA class I (SLA-2, SLA-3 and SLA-1) and three SLA class II loci (DRB1, DQB1, DQA) using PCR with sequence-specific primers (PCR-SSP) at the allele group resolution. A total of seven SLA haplotypes (Lr-5.34, Lr-7.23, Lr-31.13, Lr-56.23, Lr-56.30, Lr-59.1, Lr-65.34), comprising six unique class I and five unique class II haplotypes, were characterized in the founding animals. Class I haplotype Lr-65.0 and class II haplotype Lr-0.34 were novel; and together with Lr-56.0 these haplotypes appeared to be breed-specific. In the progeny population, Lr-7.23 and Lr-56.30 appeared to be the most prevalent haplotypes with frequencies of 34.7% and 31.6%, respectively; the overall homozygosity was 27.4%. This resource population of SLA-defined Korean native pigs will be useful as large animal models for various transplantation and xenotransplantation experiments, as well as for dissecting the roles of SLA proteins in swine disease resistance and production traits.

INTRODUCTION

The major histocompatibility complex (MHC) is a gene-dense region containing a number of important immune-related genes and is found in all jawed vertebrates (Kelley et al., 2005). The porcine MHC is divided into three genomic regions, namely class I, class II and class III, and is mapped to porcine chromosome 7, spanning the centromere with the class I and III region on the p arm and the class II region on the q arm (reviewed in Lunney et al., 2009). The porcine MHC encodes a series of membrane-bound cell surface glycoproteins called the swine leukocyte antigens (SLA) which play very important roles in the adaptive immune system. These proteins have been repeatedly implicated in swine disease resistance and vaccine response as well as tumor penetrance and production traits (reviewed in Lunney et al., 2009; Vaiman et al., 1998). In using pigs as large animal models for human transplantation and as potential xenograft donors, the SLA antigens are important targets for allo- and xeno-immunological recognition and can be directly recognized by various human immune cell subsets (Kwiatkowski et al., 1999; Shishido et al., 1997; Yamada et al., 1995). Accurate and effective SLA typing methods are crucial to study the influence of these antigens in transplant outcomes and swine immunity.

The SLA genes are highly polymorphic. The Immune Polymorphism Database (IPD)-MHC (http://www.ebi.ac.uk/ipd/mhc/sla/) currently contains 116 SLA class I alleles (44 SLA-1, 26 SLA-3 and 46 SLA-2), 159 class II alleles (82 DRB1, 44 DQB1, 20 DQA, 13 DRA) alleles, and 29 class I and 21 class II haplotypes of these loci designated by the SLA Nomenclature Committee of the International Society for Animal Genetics (ISAG) (Ellis et al., 2006; Ho et al., 2009a; Robinson et al., 2005; Smith et al., 2005a; 2005b).

A number of SLA-defined resource pig populations have been developed worldwide (Ando et al., 2005; Ho et al., 2006; 2010b; Lee et al., 2005; Sachs et al., 1976; Smith et al., 2005c; Soe et al., 2008). However, these pigs may not be readily available for researchers in other countries due to strict import/export regulations and/or exclusive usage rights. The Korean native pigs have been considered as animal models for transplantation and xenotransplantation research because of their relatively small body size (less than 70 kg at maturity) and their presumably highly inbred status due to the closed breeding program since 1986 (Kim et al., 2002). Their SLA specificities have recently been investigated by a RT-PCR, sequence-based typing (SBT) method in only a limited number of animals (Lee et al., 2008). Although

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Table 1. Specificity of the sequence-specific PCR primers for swine leukocyte antigen (SLA) class I genotyping at SLA-2, SLA-3 and SLA-1 in Korean native pigs[1].

Lane	Allele specificity[2]	Product size (bp)	Lane	Allele specificity[2]	Product size (bp)
1	SLA-2*01XX(all); SLA-1*15XX(all)/es11	256	26	SLA-3*0101/01ev04/01rh28	193
2	SLA-2*02XX(all)	139	27	SLA-3*0301/0304/03an04/03an05	149
3	SLA-2*03XX(all)/es22; SLA-1*16XX(all)/an02	134	28	SLA-3*0301~04/03an02/03an04/03an05/ 04XX(all)/hb06; SLA-1*1103	139
4	SLA-2*04XX(all)	316			
5	SLA-2*05XX(all)	127	29	SLA-3*04XX(all)/05XX(all); SLA-2*15XX(all)	207
6	SLA-2*06XX(all)	127	30	SLA-3*0502~03/05sw01	114
7	SLA-2*07XX(all)	199	31	SLA-3*06XX(all)/070102/07rh34	188
8	SLA-2*w08XX(all)	126	32	SLA-3*0602/070101~02/07Lw02	172
9	SLA-2*w09XX(all)	181	33	SLA-1*01XX(all)	209
10	SLA-2*10XX(all)	116	34	SLA-1*02XX(all)	148
11	SLA-2*11XX(all)	126	35	SLA-1*04XX(all)	181
12	SLA-2*12XX(all); SLA-1*es11	160	36	SLA-1*05XX(all)/an02	223
13	SLA-2*w13XX(all); SLA-1*1103	117	37	SLA-1*06XX(all)/1301	164
14	SLA-2*w14XX(all)/es22	150	38	SLA-1*07XX(all)	220
15	SLA-2*an04	143	39	SLA-1*08XX(all)	141
16	SLA-2*15XX(all); SLA-3*08XX(all)	203	40	SLA-1*09XX(all)	193
17	Negative control (porcine *ACTA1*)	516	41	SLA-1*w10XX(all)	180
18	SLA-3*01XX(all)	174	42	SLA-1*11XX(all)	183
19	SLA-3*0301~0304/03an02/03an04/03an05	209	43	SLA-1*12XX(all)	121
20	SLA-3*04XX(all)	192	44	SLA-1*13XX(all)	217
21	SLA-3*05XX(all)	139	45	SLA-1*rh03; SLA-2*es22	193
22	SLA-3*0601	153	46	SLA-1*16XX(all)	173
23	SLA-3*0602	140	47	SLA-1*an02	172
24	SLA-3*07XX(all)	153	48	SLA-1*cs02	209
25	SLA-3*08XX(all)	210			

[1]Primer sequences have previously been published (Ho et al., 2009b).
[2]"all" indicates all the published allele(s) that have been assigned to the group by the SLA Nomenclature Committee (Ho et al., 2009a; Smith et al., 2005b).

SBT is the most accurate method for defining SLA specificities, it can not be effectively implemented to type large swine pedigrees due to its labor-intensive and time-consuming experimental procedures (e.g. the requirement of cloning to resolve heterozygous sequences). In this study, we characterized the SLA haplotypes of six loci (SLA-2, -3, -1, -DRB1, -DQB1 and -DQA) present in a resource population of Korean native pigs using a simple and rapid PCR-sequence-specific primers (PCR-SSP)-based typing system. The information elucidated is essential for the establishment of SLA haplotype-defined Korean native pig breeding lines and subsequent selection of animals for transplant experiments.

MATERIALS AND METHODS

Animals

Eight families of Korean native pigs were originated by mating eight boars to 12 sows. The relatedness between these founding animals was not documented. This herd of pigs has essentially been maintained as a closed colony at the National Institute of Animals Science, Korea, since 1986. A total of 115 animals, including all of the 20 founding parents, 54 of their F1 progeny, and 41 of the F2 progeny derived from backcrossing, were SLA typed in this study. The breeding structure showing the F0 and F1 animals is demonstrated in Supplementary Fig. S1. Genomic DNA was extracted from blood samples at the Subtropical Animal Experiment Station, National Institute of Animal Science in Jeju Province, Korea and stored at -20°C until use.

SLA genotyping

All animals were genotyped at three SLA class I loci (SLA-2, SLA-3 and SLA-1) and three SLA class II loci (DRB1, DQB1, DQA) using methods as previously described (Ho et al., 2009b; 2010a). The typing assays relied on a set of 94 sequence-specific PCR primer pairs, which differentiated the class I and class II alleles by groups as designated by the SLA Nomenclature Committee (Ho et al., 2009a; Smith et al., 2005a; 2005b) based on shared sequence motifs (i.e. low-resolution genotyping) (Tables 1 and 2). Alleles of DRA were not characterized in this study due to their very limited polymorphism (three allele groups) and tight linkage to DRB1. Fourteen additional primer pairs were also used to confirm the allele associations in two SLA class I haplotypes (Lr-56.0 and Lr-59.0) at a higher resolution (Table 3).

Typing was set up as previously described in 10 μl reaction volumes with each PCR containing 1× PCR Gold buffer (Applied Biosystems, USA), 1× Cresol Red loading buffer (1.0

Table 2. Specificity of the sequence-specific PCR primers for swine leukocyte antigen (SLA) class II genotyping at DRB1, DQB1 and DQA in Korean native pigs.[1]

Lane	Allele specificity[2]	Product size (bp)	Lane	Allele specificity[2]	Product size (bp)
1	Negative control (porcine *ACTA1*)	516	25	DQB1*01XX (all: 0101/01be01/01ha01/01Lu01/01me03/01sh01)	165
2	DRB1*01XX (all: 0101~02); be01/ha01/ha04/Lu02	162	26	DQB1*01XX (all: 0101/01be01/01ha01/01Lu01/01me03/01sh01); sh03	180
3	DRB1*01XX (all: 0101~02); be01/ha04/me02	203	27	DQB1*02XX (all: 0201~04/02du01/02kg02/02La03/02me01/02zs16)	146
4	DRB1*02XX (all: 201/0201br05/0201du02/02du01/02du03/02ka05/02ka06/02ka08/02sp02/02sp08/02zs13)	115	28	DQB1*03XX (all: 0301~03)	166
5	DRB1*03XX (all: 0301)	180	29	DQB1*04XX (all: 040101~02/0402/0402we01/04hg09/04sk51/04sp16)	197
6	DRB1*04XX (all: 0401~04/04ga01/04ta01)	206	30	DQB1*05XX (all: 0501~03/05sp06)	193
7	DRB1*05XX (all: 0501~02/05ch01/05ka01/05ka03/05np01/05sp06)	172	31	DQB1*06XX (all: 0601~02/06sp01); zs12	204
8	DRB1*06XX (all: 0601~03Q/06sL47/06zs12)	122	32	DQB1*07XX (all: 0701)	154
9	DRB1*07XX (all: 0701/07ka03/07yo02)	133	33	DQB1*08XX (all: 0801/08ch01/08Lu03); zs13	148
10	DRB1*08XX (all: 0801/0801hg06/08hg09/08ka83/08ka92/08sp05); ka04/ka05/oj01	108	34	DQB1*09XX (all: 0901/09zh01)	146
11	DRB1*09XX (all: 0901/0901br04/09sL48/09ta01); du05/La02/oj02	105	35	DQB1*09XX (all: 0901/09zh01); Lu02	180
12	DRB1*09XX (all: 0901/0901br04/09sL48/09ta01); ka09/kb02/La02/La04	157	36	DQB1*es51/zs14	161
13	DRB1*10XX (all: 1001/10jh01/10ka06/10Lu03/10sp07); er01/La03	135	37	DQB1*02XX (0201/0204/02du01/02kg02/02me01); Lu02/zs13	193
14	DRB1*11XX (all: 1101~02/11ac21/11br02/11sp01/11zs10)	109	38	DQB1*02XX (0202/02zs16)	176
15	DRB1*w12XX (all: w12ka02/w12ka05/w12ka12)	186	39	DQB1*02XX (0201/0203/02du01/02kg02/02me01)	133
16	DRB1*13XX (all: 1301)	182	40	DQB1*02XX (0203/02zs16); 03XX (0301)	165
17	DRB1*14XX (all: 1401)	113	41	DQB1*02XX (0202/0204); zs13	173
18	DRB1*ka13	160	42	DQA*01XX (all: 0101~03/01ch01/01my01)	141
19	DRB1*ka14	134	43	DQA*02XX (all: 0201~05/0201~05/02cs01/02xu01); ka01	210
20	DRB1*kb02/kb03N/kb04N	202	44	DQA*03XX (all: 0301/03ta01/03we01)	160
21	DRB1*La03/La04/La05	197	45	DQA*04XX (all: 0401/04ta01)	124
22	DRB1*04XX (0403~04); 11XX (1101/11ac21); ka13	117	46	DQA*04XX (all: 0401/04ta01)	148
23	DRB1*04XX (0401~02/04ga01/04ta01)	160	47	DQA*w05XX (all: w05ch01)	111
24	DRB1*04XX (0401~02/04ga01/04ta01)	118	48	DQA*ka01	120

[1]Primer sequences have previously been published (Ho et al., 2010a).
[2]"all" indicates all the published allele(s) assigned to the group (Ho et al., 2009a; Smith et al., 2005a).

mM Cresol Red in 1750 mM sucrose), 2.25 mM $MgCl_2$ (Applied Biosystems), 0.25 mM of each dNTP (Genet Bio, Korea), 0.5 μg of acetylated BSA (Promega, USA), 0.5 μM each for the positive control primers (*ACTA1*, porcine α-actin) and typing primers, 0.3 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), and 25 ng of DNA (Ho et al., 2006; 2009b). A negative control was set up for each typing with water in place of DNA to check for reagent contamination (lane 17 in class I and lane 1 in class II). The cycling conditions consisted of an initial incubation of 94°C for 10 min, followed by 35 cycles of 96°C for 15 s, 65°C for 20 s and 72°C 20 s, and a final incubation of 72°C for 3 min. PCR products were electrophoresed in 2% agarose gels with 1× TAE. Interpretation of results was based on the presence of allele-specific PCR products of the expected size.

Designation of low-resolution SLA allele specificity and haplotype

The SLA allele specificities were reported with the first two digits to denote allele groups and the third and fourth digits designated "XX" to denote low-resolution group specificity (e.g. SLA-1*01XX). For class I haplotype Lr-59.0, the tentative allele SLA-2*jh02 was used directly to represent its SLA-2 specificity as this allele has not been assigned into a group due to its distinctive sequence motifs. The SLA alleles were assigned into haplotypes by pedigree which were designated with a prefix "Lr" to

Table 3. Sequence-specific primers for the confirmation of swine leukocyte antigen (SLA) class I haplotypes in Korean native pigs.

Lane	Allele specificity[1]	Haplotype specificity	Forward (F) and reverse (R) primer sequence (5′→3′)	Primer position	Product size (bp)
1	SLA-1*11XX(all)	Lr-56.0,	F: TCGACAGCGACGCCCCC	+ 186	109
		Lr-59.0	R: CTGTGCGCTGCCCATGACAC	+ 260	
2	SLA-1*11XX(all)	Lr-56.0,	F: GTGTCCCGGCCCGACC	+ 112	183
		Lr-59.0	R: CTGTGCGCTGCCCATGACAC	+ 260	
3	SLA-1* 1101~02/11mp11/11yn01	Lr-56.0	F: GGGAGCCCCGTTTCATCGAA	+ 135	164
			R: CTGTGCGCTGCCCATGACAC	+ 260	
4	SLA-2*1501; SLA-1*1101	Lr-56.0	F: GGGAGCCCCGTTTCATCGAA	+ 135	186
			R: TGTTCAGGCTCCCTCGGAAAG	+ 281	
5	SLA-2*1501/es22	Lr-56.0	F: GGACCGCGGCGGACACT	+ 486	90
			R: GGCCCTGCAGGTAGCTCCTCCA	+ 538	
6	SLA-2*1501; SLA-3*0801	Lr-56.0	F: CGACCKCAGGAAGCCCCGT	+ 135	203
			R: CGCGCAGGKTGTTCAGGC	+ 302	
7	SLA-2*1501; SLA-1*1102	Lr-56.0	F: GGAAGCCCCGTTTCATCGAA	+ 144	196
			R: CCGCGCAGGGTGTTCAGGC	+ 302	
8	SLA-2*jh02; SLA-1*rh03/st11	Lr-59.0	F: CGCACCGAAACCGAGGGA	+ 197	143
			R: GGTTGTAGTAGCCGCGCAGGT	+ 302	
9	SLA-1*w10XX(all)/1103	Lr-59.0	F: CGTGTTCCGGCCCGACCT	+ 113	190
			R: GTCATTCTGTGCGCTGCCCA	+ 266	
10	SLA-2*0301;	Lr-59.0	F: CGTGTTCCGGCCCGACCT	+ 122	147
	SLA-1*1103/1201/12hy01		R: CAATACTCCTGCCCCTCCTTCTCT	+ 228	
11	SLA-2*w13XX(all); SLA-1*1103	Lr-59.0	F: TMGARMAGGAGGGGCAGGG	+ 245	117
			R: CGGGCTCGCTCTGGTTGTAGTA	+ 322	
12	SLA-3*0301~04/03an02/03an04/	Lr-56.0,	F: TCCCCACTCCCTGAGGTATTTCG	+ 88	139
	03an05/04XX(all)/hb06; SLA-1*1103	Lr-59.0	R: CGGCTCCATCTGCGGATTG	+ 186	
13	SLA-2*jh02; SLA-1*rh03/st11	Lr-59.0	F: GCCCCGAATCCGAGGGA	+ 197	141
			R: GTTGTAGTAGCCGCGCAGGTTG	+ 300	
14	SLA-2*jh02	Lr-59.0	F: CGTGGACTCCCGCTTCCTCA	+ 142	175
			R: TCGGTAAGTCTGTGCGGTTTCCTTGTAA	+ 270	
15	Negative control (porcine *ACTA1*)		F: CGCCATGTGTGACGAAGACGAGACC	+ 21	516
			R: CACGTACATGGCGGGCACGTTGAAG	+ 384	

[1]"All" indicates all the published allele(s) that have been assigned to the group by the SLA Nomenclature Committee (Ho et al., 2009a; Smith et al., 2005b).

indicate low resolution specificities and a number for the class I haplotype followed by a number for the class II haplotype separated by a period (e.g. Lr-1.1). The low resolution haplotypes were numbered according to their high resolution equivalents when their allele group specificities were identical (e.g. Lr-5.0, SLA-2*w08XX-SLA-3*05XX-SLA-1*04XX vs. Hp-5.0, SLA-2*w08sw01-SLA-3*05sw01-SLA-1*0401). Novel haplotypes were arbitrarily numbered.

RESULTS AND DISCUSSION

A total of 115 Korean native pigs were genotyped for three SLA class I loci and three class II loci using the PCR-SSP typing panels (Tables 1 and 2). A representative gel picture showing the typing of one family (sire K5-15, dam K-17 and their offspring K6-15 and K6-16) is demonstrated in Fig. 1. The PCR-SSP typing panel did not assay five new class I alleles recently designated by the SLA Nomenclature Committee (SLA-1*1401, SLA-1*st11, SLA-1*sk13, SLA-2*jh02 and SLA-3*03pt31) (Ho et al., 2009a). In order to confirm the concordance of the allele associations in two class I haplotypes (Lr-56.0 and Lr-59.0) with their respective high resolution equivalents, 14 additional allele-specific primer pairs were used (Table 3). A representative gel picture showing the confirmation of these two haplotypes is demonstrated in Fig. 2.

Based on the inheritance and segregation of the alleles in the pedigrees, we observed seven SLA haplotypes (Lr-5.34, Lr-7.23, Lr-31.13, Lr-56.23, Lr-56.30, Lr-59.1, Lr-65.34), which comprised six unique class I haplotypes and five unique class II haplotypes (Table 4). Four of the class I haplotypes (Lr-5.0, -7.0, -56.0 and -59.0) and three of the class II haplotypes (Lr-0.1, Lr-0.13 and Lr-0.30) appeared to have high-resolution equivalents and were therefore numbered accordingly (Ho et al., 2009a; Smith et al., 2005a; 2005b). Class I Lr-56.0 was found to associate with two class II haplotypes (Lr-0.23 and Lr-0.30), while class II Lr-0.23 and Lr-0.34 were each associated with two class I haplotypes (Lr-7.0 and Lr-56.0; Lr-5.0 and Lr-65.0; respectively). Moreover, four class I haplotypes (Lr-5.0, -7.0, -31.0,

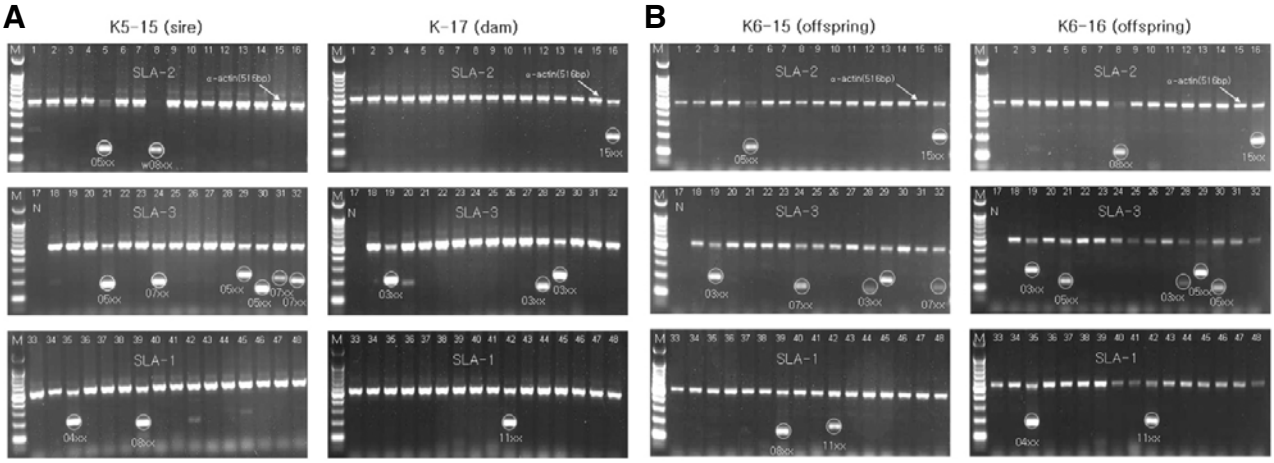

Fig. 1. Low-resolution swine leukocyte antigen (SLA) genotyping using PCR-SSP. A positive control primer pair specific for the porcine α-actin gene *ACTA1* (516 bp) was multiplexed into each reaction to check for adequate amplification (upper band). The presence of a lower (smaller) PCR product indicates the sample is positive for the allele(s) in the corresponding reaction. Specificities and the expected product size of each typing reaction are given in Table 1 for the class I typing at SLA-2, SLA-3 and SLA-1,and in Table 2 for the class II typing at DRB1, DQB1 and DQA. Examples shown here are the class I typing of a Korean native pig family consisted of (A) sire K5-15 and dam K-17, and (B) offspring K6-15 and K6-16. Sire K5-15 is heterozygous for SLA class I haplotype Lr-5.0 and Lr-7.0, with specific positive reactions in lane 5, 8, 21, 24, 29-32, 35 and 39. Dam K-17 is homozygous for SLA class I haplotype Lr-56.0, with specific positive reactions in lane 16, 19, 28, 29 and 42. Offspring K6-15 and K6-16 inherited Lr-7.0 and Lr-5.0 from the sire, respectively, and Lr-56.0 from the dam. Note the faint lower band in lane 42 of sire K5-15 and lane 20 of dam K-17 were probably a weak false positive reaction and a carry-over contamination, respectively. *M*, 100-bp PCR marker.

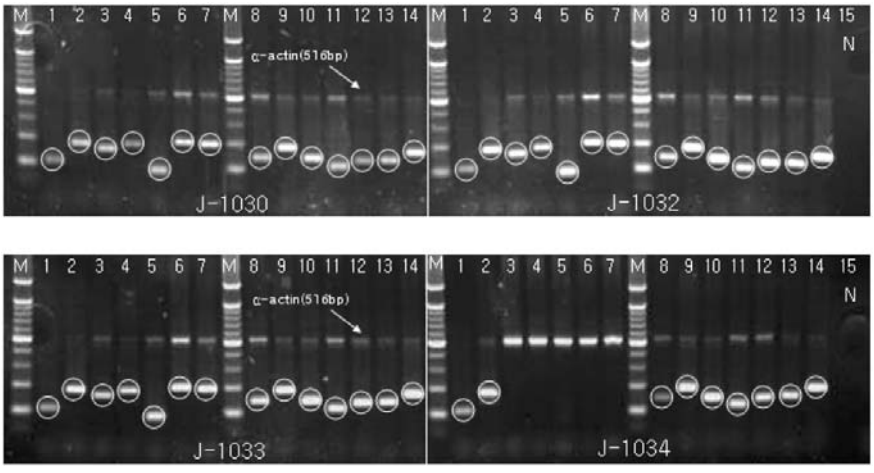

Fig. 2. Additional 14 typing reactions used initially to confirm allele associations of swine leukocyte antigen (SLA) class I haplotype Lr-56.0 and Lr-59.0 in the Korean native pigs. Primer pairs in lane 3-7 are specific for Lr-56.0; primer pairs in lane 8-11 and 12-13 are specific Lr-59.0; and primer pairs in lanes 1, 2 and 12 are specific for both Lr-56.0 and Lr-59.0. Detailed specificities and the expected product size of each reaction are given in Table 2. Examples shown here are the typing of J-1030, J-1032, J-1033 and J-1034, which are the offspring of sire K-31 and dam J-13 (Supplementary Fig. S1). J-1030, J-1032 and J-1033 are all heterozygous for Lr-56.0 and Lr-59.0 whereas J-1034 is heterozygous for Lr-7.0 and Lr-59.0. *M*, 100-bp PCR marker.

and -59.0) and three class II haplotypes (Lr-0.1, -0.13, and -0.23) have been reported previously in several outbred pig populations in the USA (Ho et al., 2009b, 2010a), while Lr-7.23, -56.30 and -59.1 were consistent with that previously characterized by SBT in three Korean native pigs. Taken together, only class I Lr-65.0 and class II Lr-0.34 appeared to be novel; these haplotypes along with class I Lr-56.0 have thus far been only described in the Korean native pig populations, indicating they may be breed-specific.

Of a maximum possible 40 (2*n*) SLA haplotypes in the 20 founding animals, we observed 13 copies of Lr-56.30, 11 of Lr-7.23, eight of Lr-65.34, five of Lr-59.1, and one each of Lr-5.34, Lr-31.13 and Lr-56.23 (Table 4). This corresponded to frequencies of 32.5%, 27.5%, 20.0%, 12.5% and 2.5% each, respectively. We also observed a similar frequency distribution of the SLA haplotypes in the progeny population with Lr-7.23 being the most prevalent haplotype (34.7%) followed by Lr-56.30 (31.6%). Twenty-six of the progeny were homozygous for their SLA genes (14 for Lr-7.23, ten for Lr-56.30 and two for Lr-65.34) which corresponded to a homozygosity of 27.4%. On the other hand, Lr-5.34 and Lr-56.23 appeared to have been bred out of the herd as these haplotypes were not detected in the F2 progeny.

Consistent with previous findings, the SLA-1 specificity of class I haplotype Lr-31.0 was not detected using the current PCR-SSP typing panel (Ho et al., 2009b). Similarly, we did not detect the SLA-3 specificity of Lr-65.0; however this haplotype was associated with both SLA-2*06XX and SLA-2*w09XX. The

Table 4. Low-resolution (Lr) swine leukocyte antigen (SLA) haplotypes identified in Korean native pigs.

SLA haplotype	Allele specificity						Haplotype frequency (%)		
	SLA-2	SLA-3	SLA-1	DRB1	DQB1	DQA	Parental animal	1st generation Progeny	2nd generation Progeny
Lr-5.34	w08XX	05XX	04XX	Lane 22*	04XX	02XX	2.5	0.9	0.0
Lr-7.23	05XX	07XX	08XX	10XX	06XX	01XX	27.5	31.5	39.0
Lr-31.13	01XX	07XX	Blank	04XX	03XX	02XX	2.5	3.7	6.1
Lr-56.23	15XX	03XX	11XX	10XX	06XX	01XX	2.5	3.7	0.0
Lr-56.30	15XX	03XX	11XX	11XX	05XX	02XX	32.5	26.9	37.8
Lr-59.1	jh02	05XX	11XX	01XX	01XX	01XX	12.5	13.9	13.4
Lr-65.34	06XX, w09XX	Blank	12XX	Lane 22*	04XX	02XX	20.0	19.4	3.7

*DRB1 of Lr-0.34 is only positive with primer pair in lane 22 which specific for DRB1*0403~04, 1101/11ac21 and ka13.

missing specificities in Lr-31.0 and Lr-65.0 were likely resulted from novel alleles with distinctive sequence motifs that were not assayed by the typing primers used. The dual SLA-2 specificities in Lr-65.0 were also likely due to a novel SLA-2 allele amplified by two different group-specific primer pairs. On the other hand, these SLA haplotypes may also posses a different number of class I loci as demonstrated in a growing number of SLA class I haplotypes (Ho et al., 2009b; 2010b; Smith et al., 2005b; 2005c; Soe et al., 2008; Tanaka-Matsuda et al., 2009).

In conclusion, we have characterized the SLA haplotypes present in a resource population of Korean native pigs. This information is essential for the development of these pigs as large animal models to study human transplantation, as well as for the understanding of the structural and functional implications of polymorphism among SLA alleles as they relate to their HLA counterparts in the development of the Korean native pigs as potential xenograft donors. The rapid, cost-effective SLA genotyping assays using PCR-SSP also represent useful tools to study SLA diversity in outbred commercial pigs and may facilitate the development of more effective vaccines or identification of disease-resistant pigs in the context of SLA antigens to improve overall swine health.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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