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Influence of swine leukocyte antigen haplotype on serum antibody titers against swine erysipelas vaccine and reproductive and meat production traits of *SLA*-defined selectively bred Duroc pigs

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ABSTRACT. We investigated possible associations of *SLA* class II haplotypes with serum antibody titers against a swine erysipelas vaccine, reproductive and meat production traits using a population of selective breeding Duroc pigs. In the selective breeding Duroc pigs, four *SLA* class II-DRB1 and -DQB1 alleles were assigned by using PCR-sequence specific primer technique. Low-resolution haplotype (Lr)-0.30 and/or Lr-0.13 were deduced from the *SLA* class II alleles in the population of *SLA*-defined Duroc pigs. *SLA*-homozygous piglets with the Lr-0.30 haplotype had relatively lower serum antibody titers against the vaccine compared to those with Lr-0.13. In contrast, there were no statistically significant differences in reproductive performance between the *SLA*-defined pigs with two *SLA* class II haplotypes. Weaning and rearing rates until the body weight of 105 kg was reached in homozygous piglets with Lr-0.30 were significantly lower than those in homozygous piglets with Lr-0.13. The *SLA*-defined pigs had lower birth and weaning weights, body weights at 60 days of age, and daily weight gains than non-selective breeding Duroc pigs. Furthermore, the *SLA*-defined pigs had slightly lower back fat thickness compared to the non-selective breeding pigs. The rib eye areas of homozygous or heterozygous pigs with Lr-0.13 were larger than those of homozygous pigs with Lr-0.30 and non-selective breeding pigs. These data suggested that *SLA* haplotypes had the potential as useful genetic markers for selective breeding in the population of *SLA*-defined Duroc pigs.

KEY WORDS: Duroc pig, reproductive and production trait, serum antibody titer, swine erysipelas vaccine, swine leukocyte antigen

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The swine major histocompatibility complex (*MHC*) is called as swine leukocyte antigens (SLA), and SLA molecules play important roles in regulation of the immune system [12]. The influences of *SLA*-encoded genes on porcine immune responses have been previously reported, including the associations between the *SLA* haplotypes and serum antibody titers against hen egg-white lysozyme [28], sheep red blood cells [2, 15], and vaccines with *Bordetella bronchiseptica* [20] and *Salmonella* typhimurium [11]. Although the *SLA* region on chromosome 7 was excluded as a candidate region affecting growth and fatness in QTL studies [8], many studies have evaluated the associations between *SLA* haplotypes and porcine economic traits other than immune responses, such as litter size, number of weaning piglets, number of ovulations, body weights, growth (daily weight gain), back fat thickness, and ham area [2, 5, 9, 12, 13, 19, 21, 30].

Recently, molecular-based procedures have been utilized for the identification of *SLA* alleles in some inbred pigs, such as NIH miniature [16], Clawn miniature swine [3], Westran pigs [10], Banna mini-pigs [33], Yucatan miniature pigs [25], Korean native pigs [4], Seoul National University (SNU) miniature pigs [32], and Microminipigs [1]. To establish an inbred pig line with

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well-characterized *SLA* haplotypes from a line of Duroc pigs, we selected a pair of Duroc pigs as the initial breeders, after which extensive breeding within progenies was carried out for nine generations. During the selective breeding procedure, we assigned two high-resolution *SLA* haplotypes (Hp), namely, Hp-27.30 (*SLA-1*06:02, SLA-1*08:02*, *SLA-2*01:02*, *SLA-3*01:01, DRB1*11:01*, and *DQB1*05:03*) and Hp-60.13 (*SLA-1*16:02*, *SLA-2*10:02*, *SLA-3*05:02*, *DRB1*04:03*, and *DQB1*03:03*), by nucleotide sequence determination of the reverse transcription polymerase chain reaction (RT-PCR) products of three *SLA* classical class I genes and two class II genes [26]. The two class I haplotypes, namely, Hp-27.0 and Hp-60.0, were novel. Selective inbreeding was performed for ten years, during which the line was developed for nine generations by repeated crossing among the sibs. The theoretical inbreeding coefficient of the selective breeding pigs was 65.5% at the ninth generation. To further investigate the associations between the *SLA* haplotypes and immune responses or economic traits, we analyzed serum antibody titers against a swine erysipelas vaccine and evaluated reproductive and meat production traits in *SLA*-defined selective breeding Duroc pigs.

MATERIALS AND METHODS

Animals and variables

The management of pigs and all procedures in the present study were conducted according to the Guideline for Animal Experiments of Gifu University. Two groups of Duroc breed pigs, namely, *SLA*-defined selective breeding (*SLA*-defined) and non-selective breeding pigs, were used in this study. The origin of *SLA*-defined Duroc pigs was the strain 'Sakura 201' derived from Duroc pigs in Japan. On the other hand, non-selective breeding pigs were produced from male and female pigs imported from the U.S.A. in Gifu and Aichi prefectures. Therefore, the genetic background might be different between *SLA*-defined and non-selective breeding pigs. For selective inbreeding of *SLA*-defined pigs, a pair of Duroc pigs was chosen as initial breeders, and substantial breeding within progenies was carried out for nine generations. The *SLA*-defined and non-selective breeding Duroc pigs were bred under the conventional condition and provided food and water *ad libitum* in the Gifu Prefectural Livestock Research Institute. A total of 62 *SLA*-defined and 76 non-selective breeding Duroc pigs were used for comparison of serum antibody titers. The *SLA*-defined pigs comprised 26 homozygous pigs with low resolution haplotype (Lr)-0.30, 29 heterozygous pigs with Lr-0.30, 0.13, and 7 homozygous pigs with Lr-0.13. Four reproductive traits, namely, gestation period, litter size, number of stillbirths, and number of suckling piglets, were compared among the 348 piglets from 50 deliveries in *SLA*-defined pigs and 128 piglets from 16 deliveries in non-selective breeding pigs. To verify the Mendelian inheritance patterns of *SLA* haplotypes in the population of selective breeding Duroc pigs, we analyzed the *SLA* class II alleles of 207 piglets that were born from 39 deliveries by mating males and females with the heterozygous Lr-0.30, 0.13 haplotype. The economic traits, namely, weaning and rearing rates, were compared among the 399 *SLA*-defined piglets and 134 non-selective breeding piglets. Body weights at birth, at weaning on 28 days of age, and at 60 days of age were measured in 399, 349, and 348 *SLA*-defined pigs and 128, 114, and 114 non-selective breeding piglets, respectively. Age upon reaching 30 kg and 105 kg body weights, daily weight gain, back fat thickness, and rib eye area were compared among the 295 *SLA*-defined pigs and 57 non-selective breeding pigs.

SLA-DRB1 and -DQB1 typing via PCR-SSP and assignment of SLA class II haplotypes

PCR-sequence specific primer (SSP) technique was performed to assign the four *SLA*-DRB1 and -DQB1 alleles to 399 pigs under the selective breeding Duroc population. Genomic DNA was extracted from peripheral blood, and subsequently PCR amplification were carried out using four DRB1 or DQB1 allele-specific primer pairs as previously described [26]. Two *SLA* class II haplotypes, namely, Lr-0.30 and Lr-0.13, were inferred based on the typing results of *SLA*-DRB1 and -DQB1 alleles in the herds. The two digit numbers after the decimal points in the assigned haplotype names represent the class II low-resolution haplotypes.

Serum antibody titers against swine erysipelas live vaccine

After confirming the disappearance of maternal antibody, pigs were inoculated with a swine erysipelas lyophilized live vaccine (Lyophilized live vaccine, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) once at 10 weeks of age. Swine peripheral blood samples were collected before and after vaccination at 10 and 20 weeks of age, respectively. Serum antibody titers against *Erysipelothrix rhusiopathiae* were measured based on a viable cell agglutination test using *E. rhusiopathiae* Marienfelde as antigen [22]. Serum antibody titers were presented as geometric means at the highest serum dilution that produces partial agglutination. The absence of natural infection of *E. rhusiopathiae* was confirmed by no-antibody titers in the sera of six pigs without the vaccination.

Reproductive and meat production traits

Gestation periods, litter sizes, and the numbers of stillbirths and sucklings were compared among three mating patterns. The haplotypes of the parents were Lr-0.30, 0.30 and Lr-0.30, 0.30 (n=9), Lr-0.30, 0.13 and Lr-0.30, 0.13 (n=31), and Lr-0.13, 0.13 and Lr-0.13, 0.13 (n=10) in *SLA*-defined pigs and non-selective breeding pigs (n=16). Rearing rates at weaning and on the day the pigs reached the 105 kg were compared among the *SLA*-defined and non-selective breeding pigs. Body weights were compared among the piglets with each *SLA* haplotype and non-selective breeding pigs. Mean body weights at birth, at weaning (28 days of age), on 60 days of age, and on the day of reaching 105 kg in piglets in the *SLA* haplotype groups were compared with those of non-selective breeding pigs. Daily weight gains (DG) were calculated upon reaching the 30-kg and 105-kg body weights. Back fat thickness and rib eye area were measured in 105 kg-body weight animals using an ultrasound diagnostic (SSD-900, ALOKA Co., Ltd., Tokyo, Japan) instrument and an image processing system (SDM-200 SCALAR Co., Ltd., Tokyo, Japan) according to the

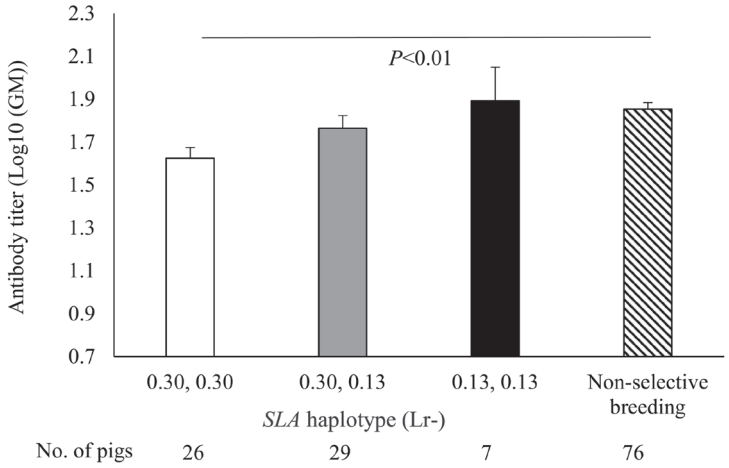

Fig. 1. Serum antibody titers against a swine erysipelas lyophilized live vaccine in Duroc pigs. The data are expressed as geometric mean (GM) of serum antibody titers and standard errors in $\log_{10}$ based values. *SLA* homozygous pigs with Lr-0.30, heterozygous pigs with Lr-0.30, 0.13, homozygous pigs with Lr-0.13, and non-selective breeding pigs are indicated by white, gray, black, and diagonal rectangles with bars, respectively. A horizontal line indicates a significant difference between the titers of SLA-defined selective breeding Duroc piglets with Lr-0.30, 0.30 and those of non-selective breeding piglets (P<0.01).

guidelines of the National Agriculture and Food Research Organization [17].

Statistical analyzes

Statistical analysis was performed by use of a computer software (Statcel-The useful add-in forms in Excel, OMS Publishing, Tokorozawa, Japan). Serum antibody titers against *E. rhusiopathiae* were presented as geometric mean and standard error (SE). Variances and differences in serum antibody titers among the two *SLA* class II haplotype groups of selective breeding Duroc pigs and non-selective breeding pigs were analyzed by the Welch's *t*-test and ANOVA and multiple group comparisons by Sheffe's F or Tukey-Kramer tests. Data on economic traits were presented as mean ± SE. Statistical differences among the two haplotype groups of *SLA*-defined Duroc pigs and non-selective breeding Duroc pigs were analyzed by one-factor analysis of variance (ANOVA) and multiple comparison (Tukey-Kramer or Steel-Dwass tests). Rearing rates upon reaching the 105-kg body weight among the three haplotype groups of *SLA*-defined Duroc pigs (Lr-0.30, 0.30, Lr-0.30, 0.13, and Lr-0.13, 0.13) and non-selective breeding pigs were evaluated by the Chi-square for independence test, using an m × n contingency table. Statistical significance was considered at P<0.05.

RESULTS

Serum antibody titers against swine erysipelas live vaccine

In a *SLA*-defined Duroc pig population, *SLA* homozygous pigs with Lr-0.30 showed lower serum antibody titers against a swine erysipelas vaccine than homozygous pigs with Lr-0.13 or heterozygous pigs with Lr-0.30, 0.13. Furthermore, *SLA* homozygous piglets with Lr-0.30 had significantly lower serum titers than non-selective breeding Duroc piglets (Fig. 1; P<0.01).

Reproductive performance

Reproductive traits were compared among the *SLA*-defined Duroc pigs with three mating patterns and non-selective breeding Duroc pigs (Table 1). We observed no statistically significant differences in the four reproductive traits among the *SLA*-defined pigs produced using three different mating patterns and non-selective breeding Duroc pigs. Nevertheless, *SLA*-defined pigs had slightly longer gestation period, slightly smaller litter sizes and number of piglets suckling piglets, and larger number of stillbirths than the non-selective breeding pigs.

As shown in Table 2, there were no statistically significant differences between the observed number of newborn homozygous piglets with Lr-0.30 or heterozygous piglets with Lr-0.30, 0.13 haplotypes and the theoretical numbers calculated based on Mendelian inheritance. By contrast, the actual number of homozygous piglets with Lr-0.13 was significantly lower than the theoretical values (P<0.05).

The weaning rates in piglets with Lr-0.30, 0.13 were significantly higher than those in homozygous piglets with Lr-0.30 (P<0.01) and non-selective breeding piglets (P<0.01) (Table 3). Heterozygous piglets with Lr-0.30, 0.13 also had higher rearing rates than those of homozygotes piglets with Lr-0.30 (P<0.01) and non-selective breeding piglets (P<0.05). Taken together, heterozygous piglets with Lr-0.30, 0.13 showed better weaning and rearing rates upon reaching 105 kg than homozygous piglets with Lr-0.30 and non-selective breeding piglets.

Table 1. Reproductive performances with mating pattern in selective and non-selective breeding Duroc pigs

Variable	Mating pattern of parents in *SLA*-defined pigs			Non-selective breeding pigs
	Lr-0.30, 0.30 and Lr-0.30, 0.30	Lr-0.30, 0.30 and Lr-0.30, 0.13	Lr-0.13, 0.13 and Lr-0.13, 0.13	
No. of deliveries	9	31	10	16
Gestation periods (day)	116.6 ± 0.34	116.2 ± 0.31	116.7 ± 0.40	113.0 ± 1.00
Litter size	7.2 ± 0.8	6.8 ± 0.4	7.2 ± 0.8	8.3 ± 3.1
No. of stillbirths	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.5	0.4 ± 0.7
No. of suckling piglets	5.8 ± 0.7	5.6 ± 0.3	6.0 ± 0.8	7.1 ± 2.6

Data are expressed as mean ± standard error. No significant differences are observed among the SLA homozygous or heterozygous pigs bearing the two haplotypes that were subjected to three different mating patterns.

Table 2. Fitness of the simple autosomal Mendelian low in 207 piglets from 39 deliveries obtained by mating between male and female *SLA* heterozygous pigs with Lr-0.30, 0.13

Variable		*SLA* class II haplotype of *SLA*-defined pigs		
		Lr-0.30, 0.30	Lr-0.30, 0.13	Lr-0.13, 0.13
No. of newborn piglets (Sum total=207)	Observed	66 (31.9)	106 (51.2)	35 (16.9)
	Expected	41 (25.0)	82 (50.0)	41 (25.0)
	P<	NS	NS	0.05

Data are expressed as number of newborn piglets (percentage). Observed: actual measurement value. Expected: calculated value from Mendel's low. NS: Not significant. The number of observed Lr-0.13-homozygous piglets was lower than the expected value.

Table 3. Weaning and rearing rates in *SLA*-defined and non-selective breeding Duroc pigs

Rate	*SLA* class II haplotype of *SLA*-defined pigs			Non-selective breeding pigs
	Lr-0.30, 0.30	Lr-0.30, 0.13	Lr-0.13, 0.13	
Weaning rate (%)	81.0 (119/147)	94.2[b,d)] (129/137)	89.6[c)] (103/115)	85.1 (114/134)
Rearing rate upon reaching 105 kg (%)	78.9 (116/147)	91.9[a,d)] (126/137)	88.7[d)] (102/115)	85.1 (114/134)

Data are expressed as percentage and the number of weaning or rearing piglets on the day of reaching 105 kg body weights. a) and b) indicate significant differences at $P<0.05$ and $P<0.01$, respectively, for non-selective breeding pigs. c) and d) indicate significant differences at $P<0.05$ and $P<0.01$, respectively, for Lr-0.30, 0.30 piglets.

Meat production traits

SLA-defined pigs had significantly lower birth and weaning weights and body weights at 60 days of age than non-selective breeding pigs ($P<0.01$; Table 4), and were older upon reaching the 30 and 105 kg body weights ($P<0.01$; Table 4). Moreover, daily weight gains of *SLA*-defined pigs were also significantly lower than those of non-selective breeding pigs ($P<0.05$). In terms of meat production traits, no significant differences were observed in ages upon reaching 30 or 105 kg weight, and daily weight gain among the three *SLA* haplotype groups. These *SLA*-defined pigs tended to have lower back fat thickness than non-selective breeding pigs. In addition to back fat thickness, rib eye areas in *SLA* homozygous or heterozygous pigs with Lr-0.13 haplotype were significantly higher than those in homozygous pigs with Lr-0.30 and non-selective breeding pigs ($P<0.05$).

DISCUSSION

MHC class I molecules trigger the immune response by presentation of peptide antigens that are derived from pathogens, including replicating viruses within cells or tumor antigens produced by cancer cells; polymorphisms in the genes encoding the class I molecules are known to influence the response to viral infection. In addition, MHC class II molecules mediate immune responses against antigens that are extracellularly generated by bacterial pathogens [18]. In this study, serum antibody titers against a live erysipelas vaccine in homozygous piglets with Lr-0.30 were found to be relatively low, followed by antibody titers in heterozygous piglets with Lr-0.30, 0.13. Pigs with the *SLA* haplotype Lr-0.30 showed slightly weaker immune responses against the erysipelas live vaccine, but serum titers were not so low as those of immune-deficient animals. Regarding immune-deficient pigs, it was reported that cellular immune responses to an attenuated human rotavirus vaccine in cloned pigs with homozygous disruption in the gene encoding immunoglobulin heavy chain were extremely lower than those in wild-type pigs [31]. The observed weaker immune responses can be influenced by the *SLA* class II alleles that constitute the Lr-0.30 haplotype. Antigenic peptides

Table 4. Comparison of productive traits among SLA-defined and non-selective breeding pigs

Variable	*SLA* class II haplotype in *SLA*-defined pigs						Non-selective breeding pigs	
	Lr-0.30, 0.30		Lr-0.30, 0.13		Lr-0.13,0.13			
	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
Birthweight (kg)	147	1.15 ± 0.02[b)]	137	1.20 ± 0.02[b)]	115	1.19 ± 0.02[b)]	128	1.36 ± 0.02
Weaning weight (kg)	119	5.40 ± 0.12[b)]	127	5.33 ± 0.11[b)]	103	5.35 ± 0.14[b)]	114	6.35 ± 0.12
Body weight on 60 days of age (kg)	118	17.84 ± 0.29[b)]	129	17.49 ± 0.31[b)]	101	17.05 ± 0.32[b)]	114	21.75 ± 0.44
Age upon reaching 30 kg body weight (day)	90	91.4 ± 12.5[a)]	109	91.3 ± 11.6[a)]	96	90.9 ± 12.5[a)]	57	74.3 ± 11.3
Age upon reaching 105 kg body weight (day)	90	202 ± 18[a)]	109	198 ± 14[a)]	96	197 ± 15[a)]	57	152 ± 12
Daily gain (g/day)	90	684 ± 104[a)]	109	703 ± 89[a)]	96	705 ± 102[a)]	57	982 ± 115
Back fat thickness (mm)	78	15.6 ± 4.3[a)]	95	15.3 ± 3.5[a)]	92	16.6 ± 4.6	57	17.9 ± 3.6
Rib eye area (cm^2)	78	41.5 ± 3.4	95	43.0 ± 3.1[a,c)]	92	43.8 ± 3.3[a,c)]	57	41.6 ± 3.0

a) and b) indicate the mean values with significant differences at $P<0.05$ and $P<0.01$ between *SLA*-defined and non-selective breeding pigs, respectively. c) indicates the mean value with significant difference of $P<0.05$ between *SLA*-defined pigs bearing the Lr-0.30, 0.30, Lr-0.30, 0.13, and Lr-0.13, 0.13 haplotypes.

derived from bacteria are known to be primarily presented through SLA class II molecules to $CD4^+$ cells by antigen-presenting cells, such as dendritic cells; $CD4^+$ helper T-cells induce B-cell production of antibodies [27]. Therefore, antibody production during the subsequent vaccination against *E. rhusiopathiae* infection might be mainly associated with *SLA* class II genotypes. In fact, Shinkai *et al.* [24] showed that non-selective breeding Duroc pigs with a specific *SLA* class II haplotype, Hp-0.50 (*DRB1*05:01*, *DQB1*02:01*) at the other breeding farm, the Shizuoka Swine and Poultry Experiment Center, Japan, showed weaker antibody responses to *E. rhusiopathiae* vaccination. The previous study also found that the antibody responses of Duroc pigs with Hp-0.13, 0.2 to the vaccination were significantly stronger than those of homozygous or heterozygous pigs with Hp-0.5, namely, pigs with Hp-0.5, 0.5 or Hp-0.5, 0.2, respectively. These previous findings were consistent with our current results, wherein *SLA*-defined homozygous pigs with Lr-0.13 and non-selective breeding Duroc pigs had higher antibody titers against the vaccine than homozygous or heterozygous pigs with Lr-0.30 and non-selective breeding Duroc pigs. Nevertheless, multiple genetic and environmental factors can influence the magnitude of immune responses, and the SLA is a potentially crucial factor influencing these responses. Taken together, the differences in *SLA* haplotypes, especially *SLA* class II haplotypes, were found to be correlated with response to *E. rhusiopathiae* vaccination, and Duroc pigs with the Lr-0.30 *SLA* class II haplotype showed weaker antibody responses to the vaccine.

Many previous association studies between *SLA* haplotypes and economic traits, such as litter size and body weights at birth and weaning, have been reported using serological SLA typing techniques so far [5, 9, 13, 19, 21]. However, in the present study, gestation periods, litter sizes, and body weights at birth and weaning were not significantly different among the three *SLA* class II haplotype groups. Therefore, the two *SLA* haplotypes, namely, Lr-0.30 and Lr-0.13, were not likely to influence the reproductive performances of the studied *SLA*-defined Duroc pigs. In our study, *SLA*-defined pigs tended to have low litter sizes and small birthweights than non-selective breeding pigs. Litter sizes in *SLA*-defined pigs, which ranged from six to eight, were slightly lower than those in non-selective breeding pigs. The *SLA*-defined pigs and non-selective breeding pigs were reared under the same condition in the same farm. Slightly smaller body sizes of *SLA*-defined pigs might influence smaller litter sizes.

In the present study, we analyzed associations between *SLA* class II haplotypes and serum antibody titers against a swine erysipelas vaccine, and evaluated reproductive and meat production traits in selective breeding Duroc pigs. However, using the class II haplotypes, we can also estimate *SLA* class I haplotypes, Hp-27.0 and/or Hp-60.0, in the selective breeding pigs. Therefore, we cannot exclude the possibility of relationship between *SLA* class I gene alleles and/or genes adjacent to the *SLA* region and some traits such as reproduction and meat production.

In the offspring cohort of heterozygous parents with Lr-0.30, 0.13, the number of newborn homozygous piglets with Lr-0.13 was lower than the expected number. Moreover, previous studies have reported that specific SLA serotypes influenced neonatal frequencies in *SLA* homozygotes, suggesting that certain haplotypes can influence embryonic survival [19]. In terms of survivability of piglets after birth, Mallard *et al.* [14] reported that NIH miniature swine with the *SLA* a/a haplotype had significantly smaller numbers of weaned piglets than those of the other two *SLA* haplotype groups, namely, the c and d haplotypes. In our study, weaning and rearing rates were slightly higher in heterozygous piglets with Lr-0.30, 0.13 than those in piglets with homozygous Lr-0.30 or Lr-0.13 haplotypes, and non-selective breeding piglets. In particular, SLA homozygosity hardly appeared to affect weaning and rearing rates in the population of *SLA*-defined Duroc pigs. By contrast, heterozygous piglets with Lr-0.30, 0.13 potentially grow normally after birth, suggesting so-called "heterosis" [7]. On the other hand, the rearing rates of homozygous piglets with Lr-0.30 were found to be a little smaller than those of heterozygous piglets with Lr-0.30, 0.13. Although the majority of causes of death in homozygous piglets with Lr-0.30 after weaning were not determined, the *SLA* haplotype, Lr-0.30, can potentially reduce survival after weaning, comparing with Lr-0.13 in our *SLA*-defined Duroc pigs. Given the weaker antibody responses against vaccination in the homozygous piglets, their higher death rates can be associated with weaker immune responses against foreign antigens, although no evidence of infectious disease was observed in this experimental farm.

Results of the present study showed that *SLA*-defined Duroc pigs had lower body weights at birth, at weaning, and on 60 days

of age and had lower daily weight gains than non-selective breeding pigs. In particular, *SLA*-defined pigs tended to have smaller bodies and slower growth rates, although differences in growth-related characteristics between two populations, the *SLA*-defined and non-selective breeding pigs, were within the range of the standard values indicated by the National Agriculture and Food Research Organization [17]. The observed phenomenon might partially reflect the impact of so-called inbreeding degeneration on the population of *SLA*-defined and inbred pigs, but the effects of the degeneration in the growth-related characteristics might be low. Furthermore, the measurements related to body growth were not associated with SLA class II haplotypes in the *SLA*-defined pigs. In our study, *SLA*-defined pigs tended to have lower back fat thickness. Moreover, *SLA* homozygous or heterozygous pigs with the Lr-0.13 haplotype had larger rib eye areas than *SLA* homozygous pigs with Lr-0.30 and non-selective breeding Duroc pigs. Therefore, the Duroc pigs with the Lr-0.13 haplotype could have more muscular bodies despite their small body sizes and slow growth rates. These characteristics were observed regardless of sex but were more prominent in males. In fact, many quantitative trait loci (QTL) and/or candidate loci on specific chromosomes except the *SLA* loci for traits related to reproductive performance have been reported so far [6, 23, 29]. Recently, one QTL study excluded the *SLA* region as a candidate region in pig chromosome 7 (SSC7) during analysis of traits affecting body growth and fatness [8]. By contrast, Wei *et al.* [30] mapped the QTL affecting fatness; back fat depth, and growth traits; daily gain and number of days to 100 kg bodyweight around the *SLA* region, suggesting the possibility of applying a *SLA* allele diversity for a marker-assisted strategy in a Meishan/Large White composite population. Moreover, in our present study, we detected a weak association between the *SLA* class II haplotype and rib eye area, which suggested that *SLA*-encoded genes or other nearby genes can potentially influence meat production traits. Although numerous other loci other than those in the *SLA* genes might have become homozygous by selective breeding over nine generations, these data suggested that *SLA* haplotypes have the potential to be useful genetic markers for selective breeding in populations of *SLA*-defined Duroc pigs. Further analyzes of the associations between *SLA* haplotypes and strength of antibody response to vaccine antigens and reproduction or meat production traits may be necessary to verify the role of the SLA complex in immune-related and economic traits using other pig breeds with various *SLA* haplotypes. Information on immunological and biological characteristics of pigs with specific *SLA* haplotypes can provide an important basis for selective breeding in pigs.

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REFERENCES

1. Ando, A., Imaeda, N., Ohshima, S., Miyamoto, A., Kaneko, N., Takasu, M., Shiina, T., Kulski, J. K., Inoko, H. and Kitagawa, H. 2014. Characterization of swine leukocyte antigen alleles and haplotypes on a novel miniature pig line, Microminipig. *Anim. Genet.* **45**: 791–798. [Medline] [CrossRef]
2. Ando, A., Shigenari, A., Kojima-Shibata, C., Nakajoh, M., Suzuki, K., Kitagawa, H., Shiina, T., Inoko, H. and Uenishi, H. 2016. Association of swine leukocyte antigen class II haplotypes and immune-related traits in a swine line selected for resistance to mycoplasmal pneumonia. *Comp. Immunol. Microbiol. Infect. Dis.* **48**: 33–40. [Medline] [CrossRef]
3. Ando, A., Ota, M., Sada, M., Katsuyama, Y., Goto, R., Shigenari, A., Kawata, H., Anzai, T., Iwanaga, T., Miyoshi, Y., Fujimura, N. and Inoko, H. 2005. Rapid assignment of the swine major histocompatibility complex (SLA) class I and II genotypes in Clawn miniature swine using PCR-SSP and PCR-RFLP methods. *Xenotransplantation* **12**: 121–126. [Medline] [CrossRef]
4. Cho, H. O., Ho, C. S., Lee, Y. J., Cho, I. C., Lee, S. S., Ko, M. S., Park, C., Smith, D. M., Jeon, J. T. and Lee, J. H. 2010. Establishment of a resource population of SLA haplotype-defined Korean native pigs. *Mol. Cells* **29**: 493–499. [Medline] [CrossRef]
5. Conley, A. J., Jung, Y. C., Schwartz, N. K., Warner, C. M., Rothschild, M. F. and Ford, S. P. 1988. Influence of SLA haplotype on ovulation rate and litter size in miniature pigs. *J. Reprod. Fertil.* **82**: 595–601. [Medline] [CrossRef]
6. Coster, A., Madsen, O., Heuven, H. C. M., Dibbits, B., Groenen, M. A. M., van Arendonk, J. A. M. and Bovenhuis, H. 2012. The imprinted gene DIO3 is a candidate gene for litter size in pigs. *PLoS One* **7**: e31825. [Medline] [CrossRef]
7. Coutellec, M. A. and Caquet, T. 2011. Heterosis and inbreeding depression in bottlenecked populations: a test in the hermaphroditic freshwater snail Lymnaea stagnalis. *J. Evol. Biol.* **24**: 2248–2257. [Medline] [CrossRef]
8. Demeure, O., Sanchez, M. P., Riquet, J., Iannuccelli, N., Demars, J., Fève, K., Kernaleguen, L., Gogué, J., Billon, Y., Caritez, J. C., Milan, D. and Bidanel, J. P. 2005. Exclusion of the swine leukocyte antigens as candidate region and reduction of the position interval for the Sus scrofa chromosome 7 QTL affecting growth and fatness. *J. Anim. Sci.* **83**: 1979–1987. [Medline] [CrossRef]
9. Gautschi, C. and Gaillard, C. 1990. Influence of major histocompatibility complex on reproduction and production traits in swine. *Anim. Genet.* **21**: 161–170. [Medline] [CrossRef]
10. Lee, J. H., Simond, D., Hawthorne, W. J., Walters, S. N., Patel, A. T., Smith, D. M., O'Connell, P. J. and Moran, C. 2005. Characterization of the swine major histocompatibility complex alleles at eight loci in Westran pigs. *Xenotransplantation* **12**: 303–307. [Medline] [CrossRef]
11. Lumsden, J. S., Kennedy, B. W., Mallard, B. A. and Wilkie, B. N. 1993. The influence of the swine major histocompatibility genes on antibody and cell-mediated immune responses to immunization with an aromatic-dependent mutant of Salmonella typhimurium. *Can. J. Vet. Res.* **57**: 14–18. [Medline]
12. Lunney, J. K., Ho, C. S., Wysocki, M. and Smith, D. M. 2009. Molecular genetics of the swine major histocompatibility complex, the SLA complex. *Dev. Comp. Immunol.* **33**: 362–374. [Medline] [CrossRef]
13. Mallard, B. A., Kennedy, B. W. and Wilkie, B. N. 1991. The effect of swine leukocyte antigen haplotype on birth and weaning weights in miniature pigs and the role of statistical analysis in this estimation. *J. Anim. Sci.* **69**: 559–564. [Medline] [CrossRef]
14. Mallard, B. A., Wilkie, B. N., Croy, B. A., Kennedy, B. W. and Friendship, R. 1987. Influence of the swine major histocompatibility complex on reproductive traits in miniature swine. *J. Reprod. Immunol.* **12**: 201–214. [Medline] [CrossRef]
15. Mallard, B. A., Wilkie, B. N. and Kennedy, B. W. 1989. Genetic and other effects on antibody and cell mediated immune response in swine

leucocyte antigen (SLA)-defined miniature pigs. *Anim. Genet.* **20**: 167–178. [Medline] [CrossRef]
16. Martens, G. W., Lunney, J. K., Baker, J. E. and Smith, D. M. 2003. Rapid assignment of swine leukocyte antigen haplotypes in pedigreed herds using a polymerase chain reaction-based assay. *Immunogenetics* **55**: 395–401. [Medline] [CrossRef]
17. National Agriculture and Food Research Organization. 2013. Japanese Feeding Standard for Swine (2013), Japan Livestock Industry Association, Tokyo) (in Japanese).
18. Neefjes, J., Jongsma, M. L. M., Paul, P. and Bakke, O. 2011. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat. Rev. Immunol.* **11**: 823–836. [Medline] [CrossRef]
19. Renard, C. and Vaiman, M. 1989. Possible relationships between SLA and porcine reproduction. *Reprod. Nutr. Dev.* **29**: 569–576. [Medline] [CrossRef]
20. Rothschild, M. F., Chen, H. L., Christian, L. L., Lie, W. R., Venier, L., Cooper, M., Briggs, C. and Warner, C. M. 1984. Breed and swine lymphocyte antigen haplotype differences in agglutination titers following vaccination with *B. bronchiseptica*. *J. Anim. Sci.* **59**: 643–649. [Medline] [CrossRef]
21. Rothschild, M. F., Renard, C., Bolet, G., Dando, P. and Vaiman, M. 1986. Effect of swine lymphocyte antigen haplotypes on birth and weaning weights in pigs. *Anim. Genet.* **17**: 267–272. [Medline] [CrossRef]
22. Sawada, T., Muramatsu, M. and Seto, K. 1979. Response of growth agglutination antibody and protection of pigs inoculated with swine erysipelas live vaccine. *Jpn. J. Vet. Sci.* **33**: 161–171 (in Japanese with English abstract).
23. Schneider, J. F., Miles, J. R., Brown-Brandl, T. M., Nienaber, J. A., Rohrer, G. A. and Vallet, J. L. 2015. Genomewide association analysis for average birth interval and stillbirth in swine. *J. Anim. Sci.* **93**: 529–540. [Medline] [CrossRef]
24. Shinkai, H., Arakawa, A., Tanaka-Matsuda, M., Ide-Okumura, H., Terada, K., Chikyu, M., Kawarasaki, T., Ando, A. and Uenishi, H. 2012. Genetic variability in swine leukocyte antigen class II and Toll-like receptors affects immune responses to vaccination for bacterial infections in pigs. *Comp. Immunol. Microbiol. Infect. Dis.* **35**: 523–532. [Medline] [CrossRef]
25. Smith, D. M., Martens, G. W., Ho, C. S. and Asbury, J. M. 2005. DNA sequence based typing of swine leukocyte antigens in Yucatan miniature pigs. *Xenotransplantation* **12**: 481–488. [Medline] [CrossRef]
26. Soe, O. K., Ohba, Y., Imaeda, N., Nishii, N., Takasu, M., Yoshioka, G., Kawata, H., Shigenari, A., Uenishi, H., Inoko, H., Ando, A. and Kitagawa, H. 2008. Assignment of the SLA alleles and reproductive potential of selective breeding Duroc pig lines. *Xenotransplantation* **15**: 390–397. [Medline] [CrossRef]
27. Spellberg, B. and Edwards, J. E. Jr. 2001. Type 1/Type 2 immunity in infectious diseases. *Clin. Infect. Dis.* **32**: 76–102. [Medline] [CrossRef]
28. Vaiman, M., Metzger, J. J., Renard, C. and Vila, J. P. 1978. Immune response gene(s) controlling the humoral antilysozyme response (Ir-Lys) linked to the major histocompatibility complexSL-A in the pig. *Immunogenetics* **7**: 231–238. [Medline] [CrossRef]
29. Wang, Y., Ding, X., Tan, Z., Xing, K., Yang, T., Wang, Y., Sun, D. and Wang, C. 2018. Genome-wide association study for reproductive traits in a Large White pig population. *Anim. Genet.* **49**: 127–131. [Medline] [CrossRef]
30. Wei, W. H., Skinner, T. M., Anderson, J. A., Southwood, O. I., Plastow, G., Archibald, A. L. and Haley, C. S. 2011. Mapping QTL in the porcine MHC region affecting fatness and growth traits in a Meishan/Large White composite population. *Anim. Genet.* **42**: 83–85. [Medline] [CrossRef]
31. Wen, K., Bui, T., Weiss, M., Li, G., Kocher, J., Yang, X., Jobst, P. M., Vaught, T., Ramsoondar, J., Ball, S., Clark-Deener, S., Ayares, D. and Yuan, L. 2016. B-cell-deficient and CD8 T-cell-depleted gnotobiotic pigs for the study of human rotavirus vaccine-induced protective immune responses. *Viral Immunol.* **29**: 112–127. [Medline] [CrossRef]
32. Yeom, S. C., Park, C. G., Lee, B. C. and Lee, W. J. 2010. SLA typing using the PCR-SSP method and establishment of the SLA homozygote line in pedigreed SNU miniature pigs. *Anim. Sci. J.* **81**: 158–164. [Medline] [CrossRef]
33. Zeng, R. and Zeng, Y. Z. 2005. Molecular cloning and characterization of SLA-DR genes in the 133-family of the Banna mini-pig inbred line. *Anim. Genet.* **36**: 267–269. [Medline] [CrossRef]