Association of swine leukocyte antigen class II haplotypes and immune-related traits in a swine line selected for resistance to mycoplasmal pneumonia

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Abbreviations

BF, back fat thickness; **BW**, body weight; **COR**, plasma cortisol concentration; **DG**, daily gain; **Hp-**, high-resolution SLA haplotype; **Lr-**, low-resolution SLA haplotype; **MHC**, major histocompatibility complex; **MPS**, mycoplasmal pneumonia of swine; **PCR-SBT**,

PCR-sequence-based typing; **SLA**, swine leukocyte antigen; **SRBC**, sheep red blood cells; **WBC**, number of white blood cells

Abstract

By selective breeding for five generations, a Landrace line has been recently established to improve resistance to mycoplasmal pneumonia of swine (MPS), daily gain (DG), back fat thickness (BF), and plasma cortisol concentrations (COR). To clarify the involvement of swine leukocyte antigen (SLA) polymorphisms in the selection process, we investigated possible associations of 11 SLA-class II haplotypes with selected traits or immune parameters. Pigs with the low-resolution SLA haplotype Lr-0.23 or Lr-0.13, which increased in frequency with the passage of generations, had less severe pathological lesions of MPS, increased leukocyte phagocytic activity, and higher white blood cell counts. In contrast, Lr-0.12 and Lr-0.2, which decreased in subsequent generations, were weakly associated with more severe pathological lesions of MPS. Therefore, in the studied Landrace line, the Lr-0.23 and Lr-0.13 haplotypes are potentially useful genetic markers for selecting and breeding animals with less severe pathological lesions of MPS.

Keywords

Swine leukocyte antigen; genotype; haplotype; disease association; mycoplasmal pneumonia

1. Introduction

Mycoplasma hyopneumoniae causes a swine enzootic pneumonia that adversely affects swine production worldwide by decreasing daily growth and feeding efficiency. The enzootic pneumonia is characterized by persistent cough and decreased appetite, resulting in significant economic losses, represented by a reduced rate of daily gain (DG) [1,2]. Thus, genetic improvement is crucial to reduce the risk of *M. hyopneumoniae* infection and increase feed conversion efficiency.

To reduce the incidence of *M. hyopneumoniae* infection and to improve meat production, a Landrace pig line was established at the Miyagi Livestock Experimental Station. This line, designated as Miyagino L2, was selected by using four criteria— DG, back fat thickness (BF), pathological changes associated with mycoplasmal pneumonia of swine (MPS), and plasma cortisol concentration (COR)—for five generations. Consequently, based on the average breeding values of Miyagino L2 in the fifth generation, the direct-selection approach for five consecutive generations has demonstrated good genetic progress for resistance to MPS and meat production traits such as DG and BF. In pigs weighing 105 kg, COR levels have a high positive genetic correlation (0.65) with MPS, suggesting good genetic advances in DG, BF, and MPS but not COR [3]. Moreover, after inoculation with sheep red blood cells (SRBCs), pigs of the selected line have lower plasma concentrations of antigen-specific antibodies and more $CD8^+$ T cells as compared with those in non-selected Landrace pigs [4]. Furthermore, after challenge with an MPS vaccine, percentages of granulocytes increased in the selected Landrace pigs compared with that in non-selected Landrace pigs. Changes in soluble factors, such as hormones and cytokines in blood, were also investigated in the selected Landrace line. In pigs of the selected line,

reduced growth hormone secretion and increased plasma IFN-γ concentrations were observed as compared with those in the non-selected one [5,6]. Detailed mechanisms of differences in these immunological phenotypes between the selected and non-selected Landrace lines remain unclear. Therefore, it is necessary to investigate possible associations between these immunophenotypes and other immunological polymorphic markers, such as major histocompatibility complex (MHC) antigens, in the selected line.

MHC molecules present non-self and self antigens to T cells and play a pivotal role in inducing antigen-specific immune responses. MHC molecules are classified into class I and class II molecules, which are encoded by class I and class II genes, respectively. The class II molecules present antigenic peptides that are derived from extracellular antigens, such as bacterial proteins [7]. Swine leukocyte antigen (swine MHC; SLA) genes are crucial for immune responses to infectious disease and vaccines [8]. Using SLA-typed pigs, the influence of SLA-encoded genes on immune and disease traits has been reported as associations between specific SLA haplotypes and antibody response levels to defined protein and vaccine antigens. Because a function of MHC class II molecules is presentation of bacteria-derived peptides [7], allelic polymorphisms of SLA class II genes may result in susceptibility or resistance to bacterial diseases, such as MPS.

To examine changes in frequencies of SLA class II haplotypes during the five generations for the selection process in the Miyagino L2 line, we have recently assigned seven DRB1 and nine DQB1 alleles in 283 individuals in the line by using a PCR-SSOP-Luminex method that we developed as a novel SLA low-resolution genotyping method. In total, 44 unique class II genotypes in a total were defined by the combination of these class II alleles in the line. In addition, low-resolution SLA class II haplotypes were deduced [9]. In the present study, to obtain more detailed allelic sequence information for the SLA class

II allele assignment at a four-digit level, we analyzed the cDNA sequences of DRB1 and DQB1 genes, which comprised 44 unique class II genotypes, in 44 individuals by using a PCR-sequence-based typing (PCR-SBT) method. Furthermore, to clarify the involvement of SLA polymorphisms in selected traits and immune factors in blood, we investigated possible associations of SLA-class II haplotypes with four selected traits; DG, BF, MPS and COR, and six immune-parameters in blood and saliva; SRBC-specific IgG, phagocytic activity of leukocytes, concentration of total IgA in saliva, number of white blood cells (WBCs), granulocyte:lymphocyte ratio, and activity of the alternative pathway of complement in pigs of the selected Landrace line.

2. Materials and methods

2.1. Animals

Landrace pigs of the Miyagino L2 line were genetically selected according to three criteria: 1) DG (in kg) calculated as [finish weight (105 kg) – start weight (30 kg)] / duration (day); 2) BF measured by ultrasound technology in animals weighing 105 kg; and 3) grade of the pathological change of MPS for five generations at the Miyagi Livestock Experimental Station from 2003 to 2008. The selection process was performed based on the breeding values of the three selection traits (DG, BF, and MPS). In addition to these three traits, plasma COR levels were adopted as a selection trait because COR levels at 105-kg body weight (BW) have a high positive genetic correlation with MPS [3]. During the selection process for the five generations, breeding values of the four selection traits were used as a sum of additive gene effects to eliminate many factors, such as environmental factors. Selection of boars and gilts for mating was conducted by using the data of the rank of aggregate breeding values that were calculated from the three selection traits as follows:

H = 0.0612DGbv – 2.71BFbv – 15.2MPSbv – 1.07CORbv, where H is the aggregate breeding value and bv is the breeding value of the trait.

The average population size of each generation was 14 boars and 39 gilts [3]. In the present study, randomly selected 283 Landrace pigs of the Miyagino L2 line were evaluated overall, comprising 63, 56, 55, 53, and 56 individuals for the first, second, third, fourth, and fifth generations, respectively. Their DNA was used for association analyses of SLA haplotypes with immune parameters in blood and saliva, SRBC-specific IgG, phagocytic activity of leukocytes, concentration of total IgA in saliva, number of WBCs, granulocyte:lymphocyte ratio, and activity of the alternative pathway of complement.

2.2. PCR amplification and DNA sequencing of two SLA class II genes, DRB1 and DQB1

To use PCR-SBT typing to assign the seven DRB1 and nine DQB1 alleles in the Miyagino L2 line pigs at the four-digit levels, we selected 44 individuals representing 44 unique class II genotypes from our previous low-resolution results from 283 pigs that were assigned by using a low-resolution typing method, PCR-SSOP-Luminex [9]. Three PCR primer pairs were used for the amplification of the second exon in the DRB1 and DQB1 loci (Table S1). PCR amplification and nucleotide sequence determination were performed as described previously [11,12]. Nucleotide sequences of the amplified products from heterozygotes for class II alleles were determined after subcloning into the pMD20 T vector (Takara Bio Inc. Otsu, Japan). At least three clones were sequenced for each allele from each locus.

2.3. Measurement of immune functions

By using whole blood obtained at 7 weeks of age and 105 kg BW, we measured phagocytic activity of leukocytes, number of WBCs, and granulocyte/lymphocyte ratio as previously reported [10]. Serum was obtained by centrifuging whole blood and used for measuring the activity of the alternative pathway of complement, concentration of IgA in saliva, and SRBC-specific IgG. The activity of the alternative pathway of complement and concentration of IgA in saliva were measured as described previously [4,10].

For measurement of SRBC-specific IgG, SRBCs (1×10^8 cells/animal) were intramuscularly injected at 70 kg BW; BW exceeded 100 kg at the second inoculation. One week after the second injection, blood samples were collected from the pigs, and serum or plasma was separated from the blood. *Mycoplasma hyopneumoniae*- and SRBC-specific IgG in the serum or plasma were detected with alkaline phosphatase-labeled rabbit polyclonal anti-pig IgG (H+L) antigen (Rockland Immunochemicals, Philadelphia, PA, USA) by using a 96-well SRBC-specific plate containing an SRBC suspension, as previously reported [10].

2.4. Measurement of plasma COR concentration

Plasma COR concentration was measured in pigs at 105 kg BW by using radioimmunoassays and enzyme-linked immunosorbent assays (Oxford Biomedical Research, Rochester Hills, MI, USA) for generations one through three and generations four and five, respectively [10].

2.5. Statistics

Frequencies between SLA-class II haplotypes in the Miyagino L2 line were evaluated by using the chi-square test. Differences in mean breeding values of the four selected traits and six immune-related phenotypes among the 11 SLA class II haplotypes were analyzed by using ANOVA and Tukey–Kramer method for multiple comparison analyses. The data were expressed as mean ± standard error. Differences were considered significant for *P* values less than 0.05.

3. Results

3.1. Nucleotide SBT of Miyagino L2 line

In total, 16 class II alleles (seven and nine alleles of the *DRB1* and *DQB1* genes, respectively) were identified in 44 pigs by SBT (Table 1). Four-digit *SLA* alleles that were assigned by SBT in the 44 pig samples were consistent with the results of the two-digit allele groups previously defined by low-resolution typing [9]. All of the class II alleles except DQB1*03XX in Lr-0.13 were assigned at the four-digit level by SBT (Table 1). In 10 individuals with Lr-0.13, we could not distinguish between DQB1*0302 and DQB1*0303 by nucleotide sequencing analysis of 241-bp amplified products in the second exon for the DQB1 gene because no nucleotide difference between the two alleles is found in the amplified products of the second exon. There were no novel allelic sequences for the seven and nine alleles of the *DRB1* and *DQB1* genes, respectively.

3.2. Haplotype frequencies in Miyagino L2 line succeeding five generations

In 283 Landrace pigs of Miyagino L2 line, 12 low-resolution SLA class II haplotypes were found by an analysis of the inheritance and segregation of the seven and

nine alleles of the *DRB1* and *DQB1* genes, respectively, in descendants of this pig population [9] (Table 1). The frequency of the 12 haplotypes in the first generation was compared with that in the fifth generation; Lr-0.23 was the most frequent haplotype in all generations. The frequency of this haplotype in the third and fourth generations markedly increased as compared with that in the second generation. The haplotype frequency of Lr-0.13 also gradually increased with the successive generations. Indeed, in the fifth generation, frequencies of these two haplotypes were slightly higher than those in the first generation (Lr-0.23: $P = 0.043$; Lr-0.13: $P = 0.085$). In contrast, the haplotype frequencies of Lr-0.12 and Lr-0.2 slightly decreased in the fifth generation as compared with the first generation (Lr-0.12: $P = 0.075$; Lr-0.2: $P = 0.042$. Fig. 1A). Furthermore, the frequencies of the other eight haplotypes were less than 0.8% in the first generation, and no significant differences in the haplotype frequencies were observed between the first and fifth generations (Fig. 1B).

3.3. Comparison of SLA class II haplotypes and selected biological and physiological traits

To clarify the relationships among SLA haplotypes and biological and physiological traits, we analyzed differences in breeding values of four selected traits among the 11 *SLA* class II haplotypes, except the haplotype with the lowest frequency, Lr-0.19 (Fig. 2). In the pigs with Lr-0.4, the mean breeding value of pathological lesions of MPS was significantly lower than those with haplotype Lr-0.2, Lr-0.12, or Lr-0.27 (Fig. 2B and Table 2; $P < 0.01$); thus, Lr-0.4 may confer resistance against the disease. In the pigs with Lr-0.13 or Lr-0.23, mean breeding values of pathological lesions of MPS were also significantly lower than those with Lr-0.2 or Lr-0.27 (Fig. 2B and Table 2; $P < 0.05$ or $P < 0.01$). In contrast, in the pigs with Lr-0.27, the mean breeding value was highest in this population,

significantly higher ($P < 0.01$ or $P < 0.05$) than those with one of the eight haplotypes except Lr-0.38 and Lr-0.2 (Fig. 2B and Table 2). Furthermore, in the pigs with haplotypes Lr-0.13 and Lr-0.23, which had less severe pathological lesions of MPS, breeding values of BF were slightly lower than those in the pigs with Lr-0.2 and Lr-0.4, respectively (Fig. 2C and Table 2). In the Miyagino L2 pigs, the mean breeding value of COR was highest in pigs with Lr-0.27 (Fig. 2D and Table 2; $P < 0.01$).

Moreover, the haplotype frequencies of Lr-0.23 and Lr-0.13, which had relatively low breeding values of pathological lesions of MPS, increased with the passage of generations. Despite the low breeding values of pathological lesions of MPS, no significant difference was observed in the frequency of the Lr-0.4 haplotype between the first and fifth generations. In contrast, the haplotype frequencies of Lr-0.2 and Lr-0.12, which were associated with more severe pathological lesions of MPS, decreased with the passage of generations (Fig. 1A and 2B). Therefore, Lr-0.23 and Lr-0.13 haplotypes may be associated with preferential selection of pigs with less severe pathological lesions of MPS. In addition, the Lr-0.23 haplotype may be associated with preferential selection of pigs with higher aggregated breeding values because of the relatively high BF and DG and low COR.

3.4. Association of SLA class II haplotypes and immune-related phenotypes

Breeding values of phagocytic activities of leukocytes in the peripheral blood were higher in pigs with any one of the five haplotypes Lr-0.1, Lr-0.38, Lr-0.13, Lr-0.14, and Lr-0.23 and lower in pigs with Lr-0.2, Lr-0.12, or Lr-0.21 (Fig. 3A, Table 3). Furthermore, three haplotypes, Lr-0.1, Lr-0.13, and Lr-0.23, were associated with a relatively large number of WBCs (Fig. 3C). In the pigs with one of those three haplotypes, the mean breeding values of the number of WBCs were significantly higher than those with Lr-0.24

(Fig. 3C, Table 3; $P < 0.01$). In contrast, in the pigs with Lr-0.4 and 0.23, activities of the alternative pathway of complement were significantly lower than those with one of the other seven haplotypes, Lr-0.1, Lr-0.21, Lr-0.2, Lr-0.13, Lr-0.12, Lr-0.24, and Lr-0.14 (Fig. 3E, Table 3; $P < 0.01$). Moreover, in the pigs with Lr-0.14, the mean breeding value of the granulocyte:lymphocyte ratio was significantly lower than those with Lr-0.12 or Lr-0.23 (Fig. 3D, Table 3; $P < 0.01$). For the other two immune-related phenotypes, concentrations of saliva total IgA and SRBC-specific IgG, no significant association was observed among those parameters and the specific haplotypes in the Miyagino L2 line (Fig. 3B and 3D). In addition, in the pigs with Lr-0.27, the breeding values of the concentrations of total saliva IgA and SRBC-specific IgG seemed to be slightly lower and higher, respectively, than those with the other 10 haplotypes, although no significant differences were observed.

4. Discussion

In the selected Landrace line, the mean breeding values of three selected traits—DG, BF, and MPS—but not COR were improved during five generations. As the generations advanced, the incidence of pulmonary MPS lesions was reduced [3]. Compared with the first generation, the percentages of pigs with Lr-0.23 or Lr-0.13 were significantly increased at the fifth generation. During the five generations, the pigs with those two haplotypes might have been preferentially selected by higher aggregated breeding values. Those pigs also had relatively low breeding values of pathological lesions of MPS; thus, Lr-0.23 and Lr-0.13 may contribute to reduced pulmonary MPS lesions. Namely, lower breeding values of MPS are interpreted to imply less severe pathological lesions of MPS.

In the Miyagino L2 population, the mean breeding value of MPS scores of animals with haplotypes Lr-0.13 and/or Lr-0.23 was –0.10, which was 0.06 lower than that of all

animals analyzed here (–0.04). The heritability of the MPS score was estimated as 0.07 [3]; therefore, the efficiency of the two haplotypes in improving MPS hepatized lesions can be estimated as approximately 0.86% throughout the entire lung. A mean phenotypic value of MPS scores of the Miyagino L2 population was 3.4% [13]. Taking these data together, we estimate that selection of these two haplotypes reduces MPS lesions by approximately 25% in the Miyagino L2 population, although this requires verification in future experiments involving actual pig populations.

Moreover, the pigs with Lr-0.4 also exhibited low breeding values of pathological lesions of MPS. However, the frequency of pigs with Lr-0.4 was 6.35% at the first generation, and only a few of the pigs with low frequencies were available for mating during subsequent generations. Hence, the percentage of pigs with the Lr-0.4 haplotype scarcely increased during the five generations. In contrast, the pigs with Lr-0.2, which significantly decreased at the fifth generation as compared with the first generation, had slightly higher breeding values of pathological lesions of MPS. Furthermore, the highest mean breeding value of DG was observed in the pigs with Lr-0.27. Despite the high value of DG, the pigs with Lr-0.27 had the highest mean breeding values of pathological lesions of MPS, as compared with those with the other 10 haplotypes; thus, Lr-0.27 may be a haplotype conferring susceptibility to *Mycoplasma hyopneumoniae* infection.

Concerning the associations between SLA haplotypes and immune-related phenotypes in the Miyagino L2 line, Lr-0.13 and Lr-0.23 were weakly associated with increased phagocytic activities and a considerable number of WBCs. There were no significant differences in the two immune-related phenotypes, phagocytic activity and number of WBCs, between the Miyagino L2 and a non-genetically selected Landrace line [4]. According to a previous study using the Miyagino L2 line, phagocytic activity

negatively correlated with pathological lesions of MPS. Accordingly, pigs with higher phagocytic activity exhibited lower morbidity states of MPS [10]. This negative correlation between phagocytic activity and morbidity states of MPS is consistent with our results of increased phagocytic activity and less severe pathological lesions of MPS in pigs with Lr-0.13 or Lr-0.23.

The activity of the alternative pathway of complement had high positive genetic correlation with pathological lesions of MPS [10]. However, we could not define the SLA haplotypes associated with activity of the alternative pathway of complement. Namely, Lr-0.4 and Lr-0.23 (significantly lower activities of the alternative pathway of complement) had less severe pathological lesions of MPS, although Lr-0.1, Lr-0.13, Lr-0.14, Lr-0.21, and Lr-0.24 (significantly increased activities of the alternative pathway of complement) had slightly less severe pathological lesions of MPS.

The concentration of SRBC-specific IgG in the pigs with Lr-0.27 was slightly higher than those with the other 10 haplotypes. The production of SRBC-specific IgG and IgM in the Miyagino L2 line is significantly lower than that in non-selected Landrace pigs [4]. In the present study, no significant difference was observed in the production of SRBC-specific IgG among the 11 SLA class II haplotypes. However, in the pigs with Lr-0.2, Lr-0.12, Lr-0.13, and Lr-0.23, the concentrations of SRBC-specific IgG were slightly lower as compared with those with Lr-0.27. Thus, the association of Lr-0.13 and Lr-0.23 with reduced pulmonary MPS lesions in the lung may also correlate somewhat with reduced production of SRBC-specific IgG.

Plasma COR levels in the pigs with Lr-0.27 were highest in those with the 11 haplotypes, suggesting a strong association between this haplotype and high COR concentration. In addition, this haplotype had the lowest aggregated breeding value in the

11 haplotypes of the Miyagino L2 line (data not shown) because of the highest degree of pathological lesions of MPS. During the selection of five generations, the breeding value of the COR level was hardly changed during the selection of five generations [3]. COR has been reported to be a humoral factor that is released at inflammatory states [14]. Furthermore, it is a negative feedback effector for the production of pro-inflammatory cytokines [15]. The heritabilities of COR level and MPS were low as compared with those of production traits [3], suggesting that the pigs with extremely high or low COR levels were not preferentially selected during five generations. Indeed, very few pigs with Lr-0.27 remained during the selection, whereas breeding values of the COR levels in all of the 10 haplotypes except Lr-0.27 were relatively low and similar to each other.

Over the past decade, influences of SLA haplotypes on many immune-related traits have been reported; these include antibody responses for SRBCs and lysozymes, parasite antigen proliferation, and bacterial phagocytosis. However, the SLA haplotypes were assigned by using serological typing techniques and analyzed for association with these immune-related traits [8, 16]. Most of these haplotypes were not equivalent to recent SLA haplotypes that were defined by SLA-DNA typing techniques and designated by the SLA Nomenclature Committee of the International Society for Animal Genetics [17]. In Miyagino L2 line pigs, we found several SLA class II haplotypes that were associated with breeding values of phagocytic activity of leukocytes, number of WBCs, granulocyte/lymphocyte ratio, and activity of the alternative pathway of complement. Further analyses of associations between SLA haplotypes and immune-related traits may be necessary for efficient selective breeding using specific SLA haplotypes in other pig breeds. Moreover, whether the association of SLA-class II haplotypes with immune-related

markers that occurs in the selected pig population, Miyagino L2, is also present in unselected pig populations remains to be clarified.

In conclusion, in a selected Landrace line, Miyagino L2, we investigated possible associations of 11 SLA class II haplotypes and immune-related traits; two specific SLA haplotypes, Lr-0.23 and Lr-0.13, were likely preferentially selected due to breeding values of physiological and immune-related traits. These SLA class II haplotypes may be a useful marker for selective breeding for less severe pathological lesions of MPS in the Miyagino L2 population.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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Figure legends

Fig. 1. SLA class II haplotype frequencies in Miyagino L2 pigs during five generations. Haplotype frequencies at each generation were calculated by χ-square test in Miyagino L2 pigs with 12 haplotypes. **A**, The *P* values for four haplotypes—Lr-0.23 ($P = 0.043$), Lr-0.13 ($P = 0.085$), Lr-0.12 ($P = 0.075$), and Lr-0.2 ($P = 0.042$)—indicate the significance of differences in haplotype frequency between the first and fifth generations. **B**, Eight low-frequency haplotypes (Lr-0.1, Lr-0.4, Lr-0.14, Lr-0.31, Lr-0.32, Lr-0.34, Lr-0.35, and Lr-0.36) show no significant differences in haplotype frequency between the first and fifth generations.

Fig. 2. Effects of SLA class II haplotypes on four traits for genetic selection in Miyagino L2 pigs.

In pigs with each SLA class II haplotype, bars indicate the mean breeding values of DG (A), pathological lesions of MPS (pathological lesions of MPS: B), BF (C), and COR (D). Black and white bars represent higher and lower mean breeding values, respectively, with significant differences among haplotypes which were assessed by multiple-comparison tests. Haplotype combinations and the mean breeding values with significant differences ($P < 0.05$ or $P < 0.01$) are indicated in Table 2. Gray bars represent mean breeding values without significant differences among haplotypes.

Fig. 3. Comparison of immune-related traits among the Miyagino L2 pigs with different SLA class II haplotypes. Bars indicate the mean breeding values of phagocytic activity of leukocytes (A), concentration of total IgA in saliva (B), number of WBCs (C), granulocyte/lymphocyte ratio (D), activity of the alternative pathway of complement (E), and SRBC-specific IgG (F) in the pigs with each SLA class II haplotype. Black and white bars represent higher and lower, respectively, mean breeding values with significant differences among haplotypes, which were assessed by multiple-comparison tests. Haplotype combinations with significant differences ($P < 0.05$ or $P < 0.01$) are indicated in Table 3. Gray bars represent mean breeding values without significant differences among haplotypes.

Table 1

SLA-class II genotypes and haplotypes deduced from DRB1 and DQB1 typing results by PCR-SSOP-Luminex and PCR-SBT in MiyaginoL2 line pigs.

No.	Low-resolution haplotypes and alleles			High-resolution haplotypes and alleles			Haplotype frequency[f]
	SLA class II haplotype	Allele specificity		SLA class II haplotype	Allele specificity		
		DRB1	DQB1		DRB1	DQB1	(%)
1	Lr-0.1	01XX	01XX	Hp-0.1	0101	0101	2.46
2	Lr-0.21	01XX	05XX	Hp-0.21	0101	0501	2.86
3	Lr-0.38	01XX	09XX	Hp-0.38	0101	0901	4.23
4	Lr-0.2	02XX	02XX	Hp-0.2	0201	0201	5.63
5	Lr-0.4[a]	02XX	04XX	Hp-0.4	0201	040101	4.93
6	Lr-0.13	04XX[b]	03XX[c]	Lr-0.13	0403	03XX[c]	21.12
7	Lr-0.19[d]	04XX	07XX	Hp-0.19	0403	0701	0.18
8	Lr-0.12	06XX	07XX	Hp-0.12	0602	0701	15.85
9	Lr-0.24	07XX	02XX	Hp-0.24	0701	0201	2.58
10	Lr-0.14	09XX	08XX	Hp-0.14	0901	0801	5.53
11	Lr-0.27	09XX	09XX	Hp-0.27	0901	0901	0.88
12	Lr-0.23	10XX	06XX	Hp-0.23	1001	0601	33.98

[a] Lr-0.4 (DRB1*02XX-DQA1*02XX-DQB1*04XX) or Lr.-0.9 (DRB1*02XX-DQA1*03XX-DQB1*04XX) (DQA1 allele is not determined in Miyagino L2 pigs.)

[b] 0403 or 0404

[c] 0302 or 0303

[d] Lr-0.19a (DRB1*04XX (0403~04)-DQA1*03XX-DQB1*07XX) or Lr-0.19b (DRB1*04XX+SR006)-DQA1*03XX-DQB1*07XX)

[e] 0501 or 05sp06

[f] Haplotype frequency represents average frequency during five generations.

Table 2

Significant differences between SLA haplotypes regarding the breeding values of selection traits.

	Haplotypes of pigs with lower breeding values				Haplotypes of pigs with higher breeding values				
Selection trait	Lr-	n	Mean	SE	Lr-	n	Mean	SE	*P* <
Daily gain	0.21	15	–4.15	16.17	0.4	27	51.99	16.17	0.05
					0.27	5	100.21	17.03	0.05
Pathological lesions of MPS	0.4	27	–0.361	0.100					0.01
	0.13	114	–0.072	0.042	0.2	33	0.188	0.129	0.05
	0.23	176	–0.184	0.040					0.01
	0.4	27	–0.360	0.100	0.12	89	–0.080	0.080	0.01
	0.1	14	–0.222	0.099					0.05
	0.21	15	–0.027	0.157					0.05
	0.4	27	–0.361	0.100					0.01
	0.13	114	–0.072	0.042					0.01
	0.12	89	–0.080	0.078	0.27	5	0.571	0.211	0.01
	0.24	14	–0.040	0.145					0.01
	0.14	30	–0.047	0.104					0.01
	0.23	176	–0.184	0.040					0.01
Backfat thickness					0.2	15	0.086	0.506	0.05
	0.13	114	–0.612	0.189	0.12	89	0.604	0.235	0.01
					0.23	176	0.264	0.142	0.05
Plasma cortisol concentration	0.1	14	0.082	0.040					0.01
	0.21	15	–0.015	0.047					0.01
	0.38	24	0.065	0.047					0.01
	0.2	33	0.132	0.043					0.01
	0.4	27	–0.034	0.054	0.27	5	0.656	0.167	0.01
	0.13	120	0.040	0.019					0.01
	0.24	14	0.033	0.031					0.01
	0.14	30	–0.009	0.039					0.01
	0.23	192	0.014	0.017					0.01

n, number of pigs; SE, standard error

P-values indicate significant differences ($P < 0.01$ or $P < 0.05$) between the SLA haplotypes with lower compared with higher breeding values of four selection traits.

Table 3

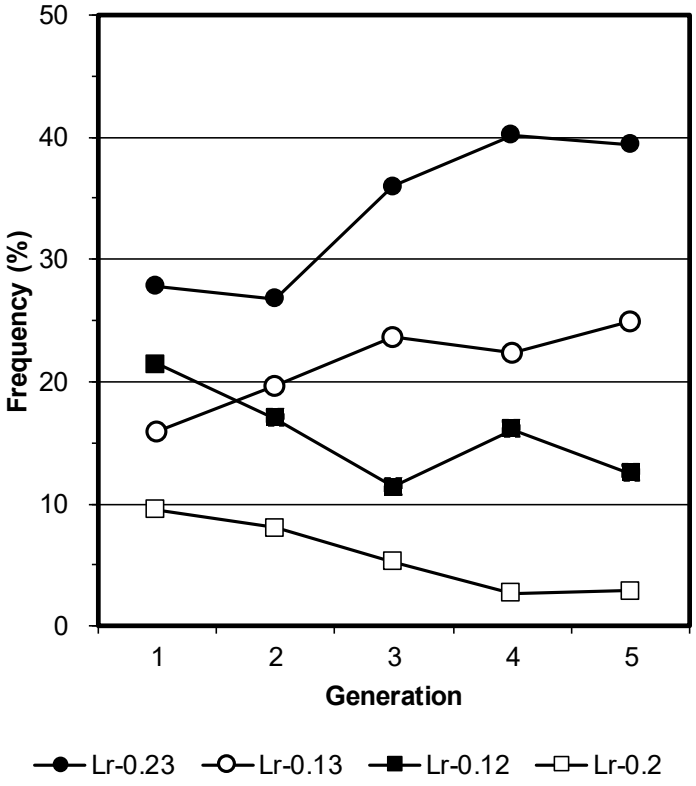
Significant differences between SLA haplotypes regarding the breeding values of immune-related traits.

	Haplotypes of pigs with lower breeding values				Haplotypes of pigs with higher breeding values				
Immune-related trait	Lr-	n	Mean	SE	Lr-	n	Mean	SE	*P* <
Phagocytic activity of lymphocytes	0.2	33	–0.484	0.127	0.1	14	0.375	0.277	0.05
					0.38	24	0.338	0.185	0.05
	0.21	15	–0.535	0.136	0.13	120	0.455	0.080	0.01
					0.23	192	0.312	0.055	0.01
	0.4	27	–0.115	0.107					0.01
	0.24	15	–0.256	0.122	0.38	24	0.338	0.185	0.05
	0.27	5	–0.395	0.335					0.01
					0.13	120	0.455	0.080	0.05
	0.2	33	–0.484	0.127	0.14	30	0.257	0.147	0.05
					0.23	192	0.312	0.055	0.01
	0.4	27	–0.115	0.107	0.13	120	0.455	0.080	0.05
	0.12	90	0.010	0.088	0.13	120	0.455	0.080	0.01
	0.24	15	–0.256	0.122	0.13	120	0.455	0.080	0.05
Number of WBCs	0.21	15	–0.048	0.024					0.05
	0.2	33	–0.036	0.014	0.1	14	0.076	0.023	0.05
	0.4	27	–0.030	0.016					0.05
					0.1	14	0.076	0.023	0.01
					0.13	120	0.018	0.010	0.01
	0.24	15	–0.120	0.015	0.12	90	–0.005	0.011	0.01
					0.14	30	–0.008	0.019	0.05
					0.23	192	0.014	0.007	0.01
Granulocyte:lymphocyte ratio	0.14	30	–0.005	0.008	0.12	90	0.031	0.006	0.05
					0.23	192	0.034	0.003	0.01
Activity of the alternative pathway of complement					0.1	14	0.008	0.005	0.01
					0.21	15	0.018	0.006	0.01
					0.2	33	0.002	0.004	0.01
	0.4	27	–0.024	0.004	0.13	120	0.003	0.002	0.01
					0.12	90	–0.001	0.003	0.01
					0.24	15	0.015	0.008	0.01
					0.14	30	0.003	0.005	0.01
					0.21	15	0.018	0.006	0.01
	0.23	192	–0.012	0.002	0.13	120	0.003	0.002	0.01
					0.12	90	–0.001	0.003	0.05
					0.24	15	0.015	0.008	0.01

n, number of pigs; SE, standard error

P-values indicate significant differences ($P < 0.01$ or $P < 0.05$) between SLA haplotypes with lower compared with higher breeding values of four immune-related traits.

A


50
40
30
20
10
0
Frequency (%)
1
2
3
4
5
Generation
Lr-0.23
Lr-0.13
Lr-0.12
Lr-0.2

B

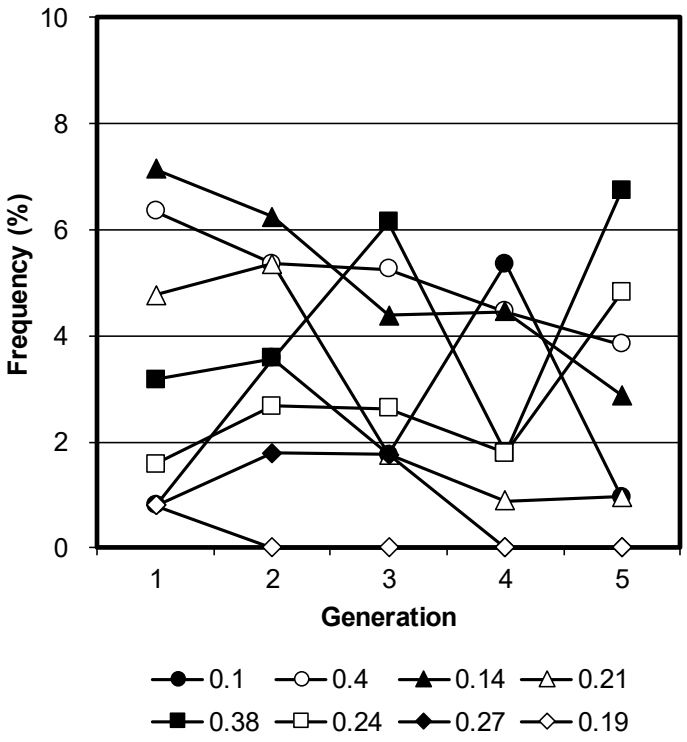

10
8
6
4
2
0
Frequency (%)
1
2
3
4
5
Generation
0.1
0.4
0.14
0.21
0.38
0.24
0.27
0.19

Figure 1

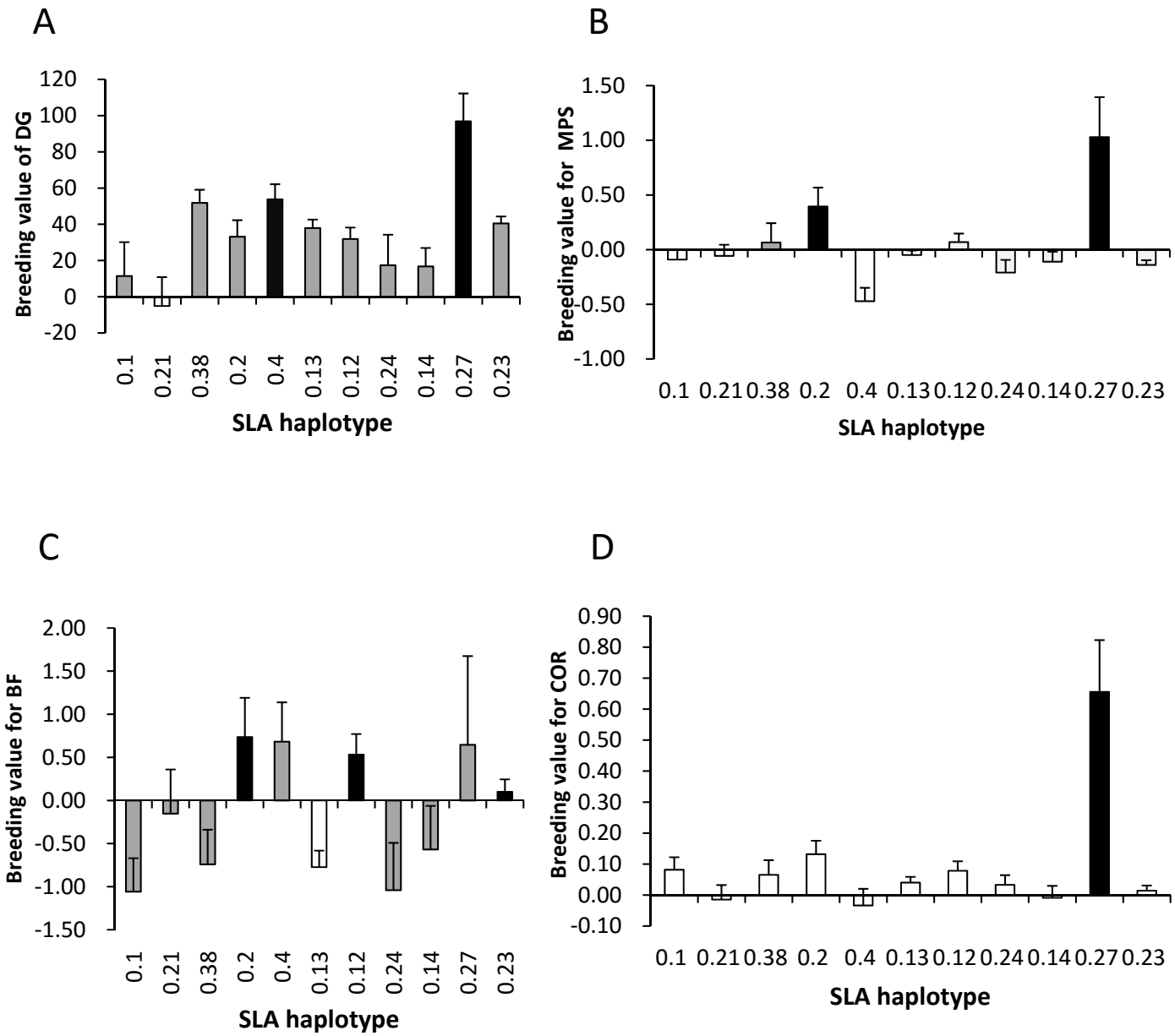

A
Breeding value of DG
120
100
80
60
40
20
0
-20
0.1
0.21
0.38
0.2
0.4
0.13
0.12
0.24
0.14
0.27
0.23
SLA haplotype
B
Breeding value for MPS
1.50
1.00
0.50
0.00
-0.50
-1.00
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype
C
Breeding value for BF
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
0.1
0.21
0.38
0.2
0.4
0.13
0.12
0.24
0.14
0.27
0.23
SLA haplotype
D
Breeding value for COR
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype

Figure 2

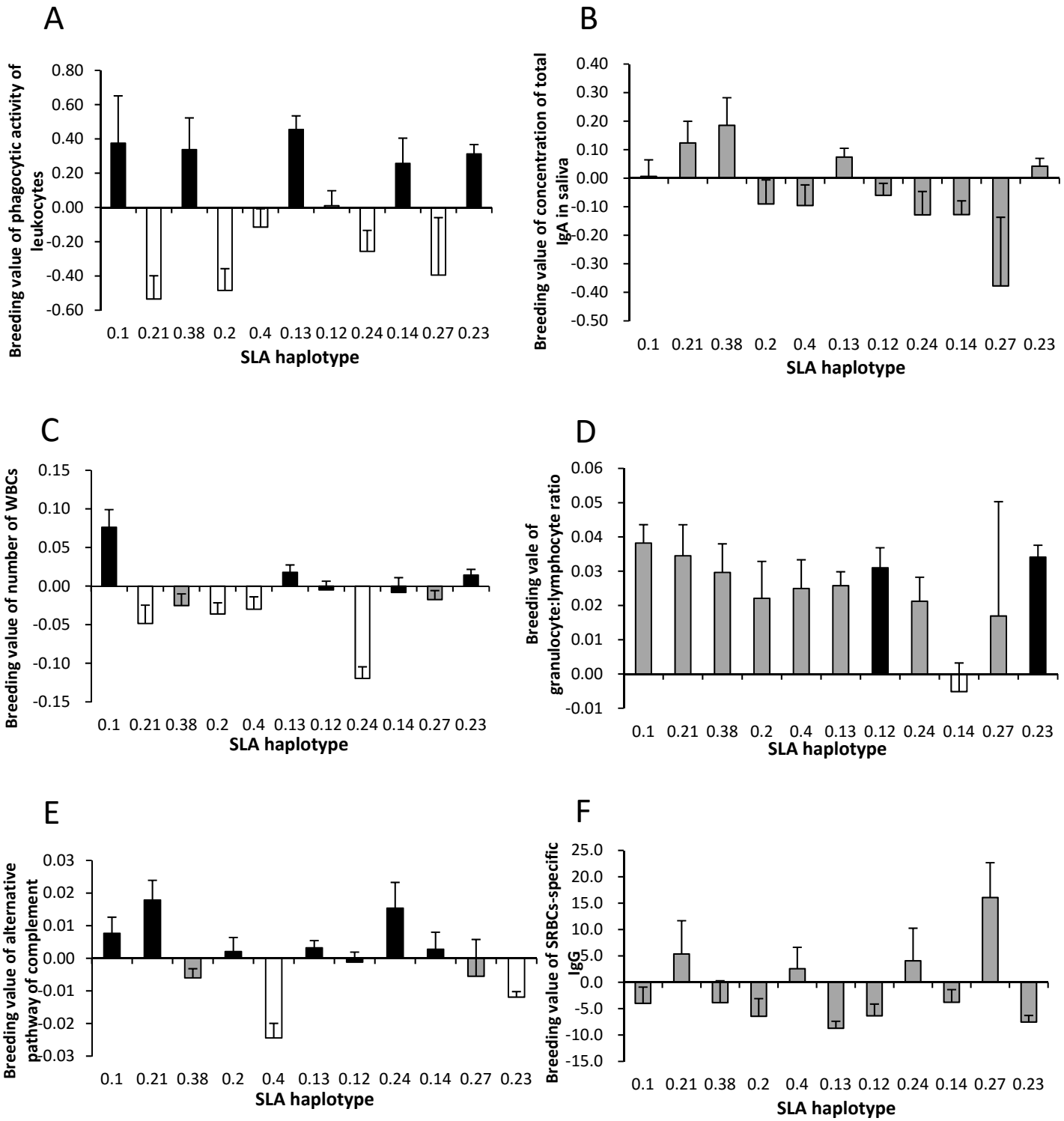

A
Breeding value of phagocytic activity of leukocytes
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype
B
Breeding value of concentration of total IgA in saliva
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype
C
Breeding value of number of WBCs
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype
D
Breeding vale of granulocyte:lymphocyte ratio
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype
E
Breeding value of alternative pathway of complement
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype
F
Breeding value of SRBCs-specific IgG
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
0.1 0.21 0.38 0.2 0.4 0.13 0.12 0.24 0.14 0.27 0.23
SLA haplotype

Figure 3

Table S1

Primer pairs used for amplification of exon 2 region of SLA DRB1 and -DQB1 loci in Miyagino L2 line.

Locus	Primer name	Forward/reverse primer sequence	Length of PCR products	Tm (°C)	Reference
DRB1	DRB-1F/DRB-02R	5'-GTCCACGCAGCGCATTTCTT-3'	247 bp	60	[9]
		5'-GGTAGTTGTGTCTGCA-3'			This study
DQB1	DQB-2F/DQB-3R	5'-TCCAGTTTAAGGGCGAGTGCTA-3'	241 bp	54	[12]
		5'-GTGCCTTCCTCTATCTGGTAGT-3'			[12]
	DQB-6F/DQB-3R	5'-ACCAGTTTAAGTTCGAGTGCTAC-3'	241 bp	54	[9]
		5'-GTGCCTTCCTCTATCTGGTAGT-3'			[9]