Genetics and Molecular Research

Polymorphism and haplotype analyses of swine leukocyte antigen *DQA* exons 2, 3, 4, and their associations with piglet diarrhea in Chinese native pig

X.Y. Huang[1], Q.L. Yang[1], J.H. Yuan[1] and S.B. Gun[1,2]

[1]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China
[2]Gansu Research Center for Swine Production Engineering and Technology, Lanzhou, China

Corresponding author: S.B. Gun
E-mail: gunsb@gsau.edu.cn

Genet. Mol. Res. 14 (3): 10461-10472 (2015)
Received January 31, 2015
Accepted May 13, 2015
Published September 8, 2015
DOI http://dx.doi.org/10.4238/2015.September.8.7

ABSTRACT. In this study, 290 Chinese native Yantai black pig piglets were investigated to identify gene polymorphisms, for haplotype reconstruction, and to determine the association between piglet diarrhea and swine leukocyte antigen (SLA) class II *DQA* exons 2, 3, and 4 by polymerase chain reaction-single stranded conformational polymorphism and cloning sequencing. The results showed that the 5, 8, and 7 genotypes were identified from *SLA-DQA* exon 2, 3, and 4, respectively, based on the single-stranded conformational polymorphism banding patterns and found a novel allele *D* in exon 2 and 2 novel mutational sites of allele *C* (c.4828T>C) and allele *F* (c.4617T>C) in exon 3. Polymorphism information content testing showed that exon 2 was moderately polymorphic and that exons-3 and -4 loci were highly polymorphic. The piglet diarrhea scores for genotypes AB (1.40 ± 0.14) and AC (1.54 ± 0.17) in exon 2, AA (1.22 ± 0.32), BC (1.72 ± 0.13), DD

(1.67 ± 0.35), and CF (1.22 ± 0.45) in exon 3, and AD (2.35 ± 0.25) in exon 4 were significantly higher than those for the other genotypes ($P \leq 0.05$) in *DQA* exons. There were 14 reconstructed haplotypes in the 3 exons from 290 individuals and *Hap12* may be the diarrhea-resistant gene. Haplotype distribution was extremely uneven, and the *SLA-DQA* gene showed genetic linkage. In this study, we identified molecular genetic markers and provided a theoretical foundation for future pig anti-disease resistance breeding.

Key words: DQA gene; Gene polymorphism; Haplotype reconstruction; Piglet diarrhea; Swine leukocyte antigen

INTRODUCTION

The swine major histocompatibility complex (MHC) or swine leukocyte antigen (*SLA*) complex is located on chromosome 7 (SSC7p1.1-1q1.1), spanning the centromere, and comprising the class I, II, and III regions (Kelley et al., 2005; Renard et al., 2006). *SLA* class II includes heterodimeric proteins composed of 2 non-covalently linked α (34 kDa) and β (29 kDa) chains with α chain coded by the *DRA* and *DQA* genes (Lunney et al., 2009; Takeshima et al., 2009). The α and β domains together form the peptide-binding region, where molecular pockets influence the binding of foreign peptides and subsequent immune responses (Vander et al., 1990). In addition, *SLA* class II molecules are cellular glycoproteins involved in antigen presentation to CD4+ T lymphocytes and are very polymorphic in both the *DR* and *DQ* genes (Lechler et al., 1991; Escayg et al., 1996; Patch et al., 2011).

The S*LA-DQA* gene consisting of 4 exons and 3 introns is approximately 5.5 kb encoding 255 amino acids (Bao et al., 2012) and is a cluster of genes responsible for antigen presentation and a highly polymorphic genomic region in vertebrates that has become a popular functional marker system for studying adaptive variation. The *SLA-DQA* gene plays important roles in immune responsiveness and disease resistance (susceptibility) (Gautschi and Gaillard, 1990; Ho et al., 2010). Variation in genes may affect immune responses to pathogens, leading to variation in disease susceptibility (Racioppi et al., 1991). Bao et al. (2012) found that *SLA-DQA* was broadly expressed in 11 piglet tissues, which may enhance humoral immunity and cell immunity to reduce signal transduction of F18 bacteria, and improved disease resistance. Yang et al. (2013, 2014) found that the various *DQA* and *DRA* genotypes significantly influenced the piglet diarrhea. Exploring the genetic influence on animal diseases can be used to effectively identify and control livestock diseases to reduce disease costs and increase animal health (Mallard et al., 1991; Eveline et al., 2008).

Piglet diarrhea death is a leading factor affecting the economic benefits of the swine industry, and has a complex etiology and high fatality, making it difficult to control. According to previous reports, microorganisms have been associated with pig neonatal enteritis, including rotavirus and coronavirus. Further, the enterotoxigenic *Escherichia coli* (ETEC) and *Clostridium perfringens* type C, among other bacteria, interact consistently in a synergistic and competitive manner. Disturbances in this microbial community can cause bacterial overgrowth and disease (Wei, 2004; Maddox-Hyttel et al., 2006), and thus piglet diarrhea is one of the primary diseases affecting piglet survival. Some studies have confirmed that post-weaning

diarrhea and edema disease were mainly caused by the ETEC F18 pathogen (Nagy and Fekete, 1999). Yantai black pig is a Chinese native pig of the Shangdong Province local gray skin pig. It is a good local pig resource to China with high growth performance, resistance of forage, high prolificacy, and strong disease resistance and has become a recognized healthy and safe meat, playing an important role in the economic development of the Chinese pig industry.

Relatively little is known about *DQA* exons 2, 3, and 4 in Chinese native Yantai black pig; therefore, we identified the *SLA* class II gene to analyze sequence variation, genetic haplotype, linkage disequilibrium (LD), and the relationship between gene and post-weaning piglet diarrhea, which has not been previously reported for piglet diarrhea.

MATERIAL AND METHODS

Samples collection and DNA extraction

Ear tissue samples were collected randomly from 290 Yantai black pig piglets from a pig breeding farm in Honggu Region, Gansu Province of China. These piglets were born during June to October 2013, and the fodder, feeding conditions, and disease control during the experiment were in accordance with the Guide for Chinese Feeding Standard of Swine. Piglet diarrhea was recorded twice per day from birth to weaning (0-28 days) and was assigned a daily score based on a standard evaluation of symptom traits. Evaluation standards were as follows: 0: normal, manure solid feces; 1: slight diarrhea, manure soft and loose feces; 2: moderate diarrhea, manure semi-liquid feces; and 3: severe diarrhea manure liquid and unformed feces (Kelly et al., 1990; Cooper and Helman, 2000). The pig ear tissue samples were preserved in 5 mL alcohol in tubes and immediately transported to the laboratory on ice before DNA isolation. Genomic DNA was extracted from the ear tissues samples using a standard phenol-chloroform extraction procedure (Sambrook and Russell, 2001) and stored at -20°C.

Polymerase chain reaction (PCR) amplification and single-stranded conformational polymorphism (SSCP) analysis

Three pairs of primers were designed to amplify the exon 2 (378 bp), exon 3 (388 bp), and exon 4 (278 bp) fragments of *SLA-DQA* (GenBank accession No. AY 303988) using the Primer 5.0 online software. Primer sequences are shown in Table 1.

Table 1. Primer and SSCP condition of *SLA-DQA* exon 2, 3, and 4 nucleotides.

Region	Primer (5'→3')	Reference sequences	SAF	AT (°C)	SSCP condition
Exon 2	F: GTCAAGTTCTCTTGTCACT R: TGTGAACGGGTAGATTCTGT	GenBank accession No. AY 303988	378 bp	55.5	250 V 30 min; 170 V 20 h
Exon 3	F: CAGGACACAACGCAGAACTCA R: GAAACAAAGGTTCCAAAAGGG		388 bp	56.0	250 V 30 min; 200 V 20 h
Exon 4	F: CCGCTCTGTGTTCTGACTTCTAC R: GCCTCCTCCCACTTTCCTTC		278 bp	56.2	250 V 30 min; 200 V 23 h

AT = annealing temperature; SAF = size of amplification fragment.

PCR amplifications were performed in a 25-μL final volume and consisted of 10X PCR buffer (including 15 mM Mg^{2+}), 50 to 100 ng genomic DNA, 10 pmol of each primer, 2.5

mM dNTPs, 5 U *Taq* DNA polymerase (TaKaRa, Shiga, Japan), and ddH_2O to make up the final volume. PCR amplifications consisted of 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 30 s at annealing temperature as shown in Table 1, 72°C for 30 s, and final extension at 72°C for 10 min.

The PCR products of the *SLA-DQA* genes were detected by 2% agarose gel electrophoresis (Biowest Agarose, Nuaillé, France) using 1X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA, pH 8.0). The gels were stained with nucleic acid fuel (BioTeKe, Beijing, China) and photographed under UV light (1000X Press, Syngene, Cambridge, UK).

The SSCP analysis of the *SLA-DQA* genes was performed using a 3 μL aliquot of each SSCP parent mixed with 7 μL denaturing solution (98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 20 mM EDTA, pH 8.0). After heat denaturation at 98°C for 10 min, the samples were immediately chilled on ice for 10 min to prevent heteroduplex formation and then loaded onto 21 x 22 cm 10% acrylamide:bis-acrylamide gels (39:1 acrylamide:bis-acrylamide). Electrophoresis was performed in 1X TBE under the conditions described in Table 1. The polyacrylamide gel was visualized by silver staining after electrophoresis (Sanguinetti et al., 1994).

Cloning and sequencing

Compared with the SSCP banding patterns, the PCR products of different representative banding patterns were purified using the DNA Fragment Quick Recover Kit (Tiangen, Beijing, China) and cloned into the pMD19-T vector (TaKaRa, Shiga, Japan). When the PCR-cloned products of different representative banding patterns were capable of recovering the banding patterns as the original PCR products, representative clones were selected from 2 separate PCR samples and subjected to sequencing by Sangon Biotech Co., Ltd. (Shanghai, China).

Statistical analysis

Sequence alignments and translations were carried out using MEGA5 software (Tamura et al., 2011). The identified single-nucleotide polymorphisms (SNPs) were compared with the swine NCBI dbSNP database using BLAST. The frequencies of genotypes and alleles, observed heterozygosity (H_O), and effective number of alleles (N_E) were calculated using POPGENE (version 1.32); the polymorphism information content (PIC) was calculated using Botstein's method (Botstein et al., 1980). The PHASE v2.11 (Stephens and Donnelly, 2003) software based on Bayesian algorithm was used to build haploid types. The SHEsis software (http://analysis.bio-x.cn/SHEsisMain.htm) was used to calculate the LD value.

The effects of genotype on piglet diarrhea were estimated using the general linear mixed-effect models procedure in the SPSS 18.0 software (SPSS, 2008), according to the following statistical model:

$$Y_{ijk} = \mu + B_i + G_j + S_k + e_{ijk}$$

where Y_{ijk} = the observed piglet diarrhea score, μ = mean of the population, B_i = effect of the season (i = 1 for summer or 2 for autumn), S_k = fixed effect of sex (k = 1 for male or 2 for female), Gj = effect of the genotype (j = 1, 2, 3), and e_{ijk} = the random error.

RESULTS

Genetic polymorphism analysis of *SLA-DQA* exons 2, 3, and 4

There were 5, 8, and 7 genotypes identified from exons 2, 3, and 4, respectively, based on the SSCP banding patterns (Figure 1). The genotype frequencies and allele frequencies of exons 2, 3, and 4 in Yantai black pig were presented in Table 2. There were 4 alleles (*A*, *B*, *C*, and *D*) and 5 genotypes (AA, BB, AB, AC, and AD) detected in exon 2; allele *A* was the dominant allele with a frequency of 0.71 (Figure 1A). The 4 alleles were described as: *A* (G-A-G-T-G-C-A-C-A-C-T-T-A-A-T-G-C-A), *B* (T-A-T-C-A-C-A-C-G-T-G-G-T-C-C-C-A-A), *C* (G-T-G-C-G-T-G-T-A-C-T-T-A-A-C-G-C-A), and *D* (T-A-G-C-G-T-G-T-G-C-T-T-A-C-C-G-A-A). For exon 3, 8 genotypes (AA, BB, AB, CC, BC, DD, EE, and CF) were identified and formed by 6 alleles (*A*, *B*, *C*, *D*, *E*, and *F*) and allele *B* was predominant with a frequency of 0.56 (Figure 1C). All alleles were described as: *A* (T-T-G-A-C-A-G-A-T), *B* (T-T-A-G-C-A-G-G-T), *C* (T-C-G-A-C-A-G-A-C), *D* (T-T-A-G-X-X-X-A-T), *E* (T-C-G-A-C-A-G-A-T), and *F* (C-C-G-A-C-A-G-A-T) (X represented nucleotide deficiency). A total of 4 alleles (*A*, *B*, *C*, and *D*) and 7 genotypes (AA, BB, AB, CC, BC, AD, and BD) were detected in exon 4 and allele *A* with a frequency of 0.55 was the predominant allele among the 4 alleles (Figure 1B). The 4 alleles were described as *A* (A-A-G), *B* (A-A-A), *C* (A-T-G), and *D* (G-T-G).

According to the protocol of Vaiman et al. (1994), the genetic polymorphic indexes of H_O, N_E and PIC indicated that the exon 2 locus was moderately polymorphic (PIC < 0.50), which was in accordance with the results of Yang et al. (2013); exon 3 and 4 loci were highly polymorphic in the Yantai black pig populations (PIC > 0.50) (Table 2).

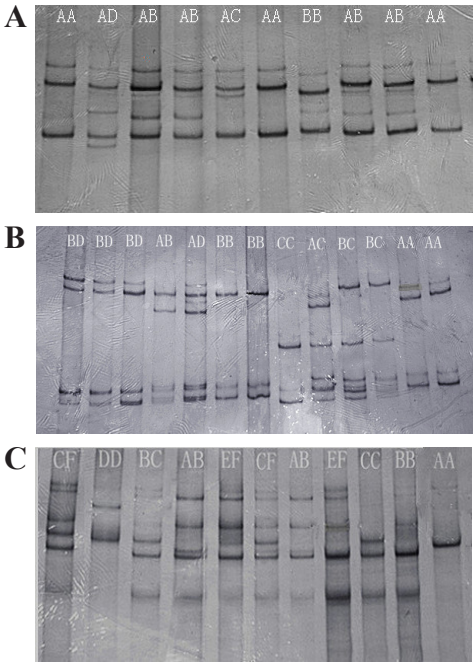

Figure 1. SSCP analyses of *SLA-DQA* exon 2 (**A**), 3 (**C**), and 4 (**B**) nucleotides in Yantai black pig.

Table 2. Allelic and genotypic frequencies, population genetic parameters of SNPs of *SLA-DQA* exons 2, 3, and 4 in Yantai black pigs and their allele accession Nos.

Locus	Allele				Genotype		N_E	H_O	PIC
		GenBank accession No.		Frequencies		Frequencies			
Exon 2	A	Known	KM411971	0.71 (412)	AA	0.46 (133)	0.39	1.65	0.43
	B	Known	KM411972	0.15 (87)	BB	0.04 (12)			
	C	Known	KM411973	0.08 (46)	AB	0.22 (64)			
	D	New	KM411974	0.06 (35)	AC	0.16 (46)			
					AD	0.12 (35)			
Exon 3	A	Known	KM485555	0.09 (50)	AA	0.04 (12)	0.38	2.63	0.58
	B	Known	KM485556	0.56 (325)	BB	0.40 (116)			
	C	New	KM485557	0.15 (87)	AB	0.10 (29)			
	D	Known	KM485558	0.04 (23)	CC	0.02 (6)			
	E	Known	KM485559	0.15 (87)	BC	0.23 (67)			
	F	New	KM485560	0.01(6)	DD	0.04 (10)			
					EE	0.15 (44)			
					CF	0.02 (6)			
Exon 4	A	Known	KM485561	0.55 (319)	AA	0.47 (138)	0.39	2.55	0.54
	B	Known	KM485562	0.28 (162)	BB	0.18 (54)			
	C	Known	KM485563	0.11 (64)	AB	0.08 (22)			
	D	Known	KM485564	0.06 (35)	CC	0.08 (24)			
					AD	0.07 (20)			
					BC	0.06 (16)			
					BD	0.06 (16)			

N_E = effective number of alleles; H_O = observed heterozygosity; PIC = polymorphism information content.

Nucleotide and amino acid variation of *SLA-DQA* exons 2, 3, and 4

In this study, a total of 14 alleles at 3 *SLA-DQA* loci were identified in 290 Yantai black pigs. Compared with reference sequence, the DNA sequencing of the amplified products of the 3 genes included exon and intron fragments; only the exon sequence fragments are shown in Figure 2. According to Figure 2, 20 SNPs, 9 SNPs, and 3 SNPs were detected in exons 2, 3, and 4, respectively; these results indicated that the 3 exons had abundant polymorphism. When compared with the pre-exiting *SLA-DQA* allele sequences (NCBI BLASTn: http://blast.ncbi.nlm.nih.gov/Blast.cgi), allele *D* with 7 SNPs in exon 2, allele *C* with the new SNP site c.4828T>C, and allele *F* with the new SNP site c.4617T>C in exon 3 were identified for the first time. All alleles' complete coding sequences and protein sequences were submitted to the GenBank database. Official allele designations assigned by the ISAG SLA Nomenclature Committee are shown in Table 2. Nucleotide mutations lead amino acid variation; 3 SNPs of allele *D* in exon 2 lead to 3 amino acid conversions of proline (Pro) to serine (Ser), threonine (Thr) to alanine, and asparagine to Thr; 2 SNPs including c.4618T>C and c.4828T>C in exon 3 caused amino acid conversion of Ser to Pro and cysteine (Cys) to arginine, respectively, which were missense mutations.

Correlating genotypes analyses on piglet diarrhea

Statistical analysis showed that the genotype significantly affected piglet diarrhea at the swine *DQA* exon 2, 3, and 4 loci ($P < 0.05$) (Table 3), and the influences of the season and sex in 3 exons were not significant ($P > 0.05$) (Table 3).

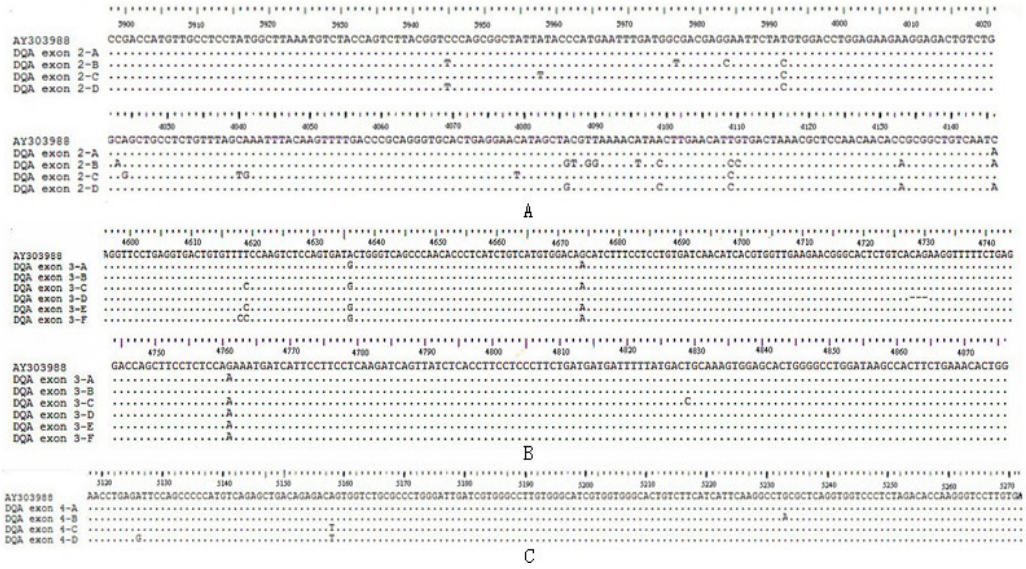

Figure 2. Alignment of *SLA-DQA* exon 2 (**A**), 3 (**B**), and 4 (**C**) allele nucleotide sequences compared to the reference sequence AY303988.

Table 3. Effects of different factors on piglet diarrhea scores in *DQA* exon 2, 3, and 4 genes.

Factors	Exon 2			Exon 3			Exon 4		
	Season	Sex	Genotype	Season	Sex	Genotype	Season	Sex	Genotype
F	3.075	1.733	34.141	2.782	0.042	55.201	1.452	0.301	42.461
P	0.079	0.188	0.001*	0.095	0.838	0.001*	0.228	0.583	0.001*

*Significant at 0.01 level.

According to the genotypic linear contrasts, the genotype diarrhea scores of the 3 exons were significantly different (Table 4). In exon 2, the genotypes AB (1.40 ± 0.14) and AC (1.54 ± 0.17) showed higher diarrhea scores than the genotypes AA (0.64 ± 0.09), BB (0.40 ± 0.43), and AD (0.92 ± 0.20). In exon 3, individuals with genotypes AA (1.22 ± 0.32), BC (1.71 ± 0.13), DD (1.67 ± 0.35), and CF (1.22 ± 0.45) showed higher diarrhea scores than other genotypes. In exon 4, the diarrhea scores of genotypes AD (2.35 ± 0.25) were significantly higher than the other genotypes ($P < 0.05$) (Table 4). The animals with higher diarrhea score genotypes were excluded for artificial disease-resistant varieties breeding because these individuals showed a higher incidence of diarrhea.

LD and haplotype analyses

The haplotype construction and its association with piglet diarrhea in Yantai black pig are shown in Table 5. The results of haplotype analysis of all SNPs showed that 14 haplotypes were identified in 290 individuals (all haplotype frequencies <0.02 were ignored in analyses). In Table 5, Hap1 and Hap2 showed the highest frequencies with Hap1 (23.62%) and Hap2 (13.79%) in Yantai black pig. Hap8 and Hap12 showed the lowest frequencies with Hap6

(2.07%) and Hap8 (2.07%). The sums of all haplotype frequencies were 77.23%, which were the most frequent haplotypes. The low-frequency haplotypes were likely rare haplotypes. Hap4 and Hap8, which were significantly higher than the controls ($P < 0.05$), may indicate susceptibility to piglet diarrhea. Hap2 and Hap6, which were the controls, were significantly higher than the cases ($P < 0.05$), and may be disease-resistant haplotypes. Hap10 was only detected in the controls and not in the cases, indicating that Hap10 was strongly disease-resistant.

The result of LD tests between all SNPs in 3 exons discovered that the D' value ranged from 0.087 to 0.271; the r^2 value ranged from 0.002 to 0.023. This indicated that the SNPs of exon 2, 3, and 4 had little LD. The reason may be that the haplotype recombination would be high and LD would be low in genovariation-dense regions.

Table 4. Least square means and standard errors of the piglet diarrhea scores of different genotypes in the *DQA* exon 2, 3, and 4 genes.

Locus	Exon 2		Exon 3		Exon 4	
Genotype	SN	LSM ± SE	SN	LSM ± SE	SN	LSM ± SE
AA	133	0.64 ± 0.09^{Ba}	12	1.22 ± 0.32^{A}	138	0.75 ± 0.09^{Bb}
BB	12	0.40 ± 0.43^{B}	118	0.70 ± 0.10^{B}	54	0.79 ± 0.15^{Bb}
AB	64	1.40 ± 0.14^{A}	6	0.33 ± 0.22^{B}	24	1.02 ± 0.24^{ab}
AC	46	1.54 ± 0.17^{A}	0	0	0	0
AD	35	0.92 ± 0.20^{a}	0	0	20	2.35 ± 0.25^{Aa}
CC	0	0	26	0.78 ± 0.45^{B}	22	1.45 ± 0.23^{ab}
BC	0	0	64	1.71 ± 0.13^{A}	16	0.92 ± 0.29^{Bb}
DD	0	0	10	1.67 ± 0.35^{A}	0	0
BD	0	0	0	0	16	1.27 ± 0.28^{ab}
EF	0	0	44	0.75 ± 0.16^{B}	0	0
CF	0	0	6	1.22 ± 0.45^{A}	0	0

SN indicates the sample number; LSM ± SE indicates the least square means ± standard errors. A and B in the same row mean significant difference ($P < 0.01$); a and b mean significant difference ($P < 0.05$); same letters mean not significant difference ($P < 0.05$).

Table 5. *SLA-DQA* gene haplotype reconstruction and its association with piglet diarrhea.

Name	Haplotypes	Frequencies (%)	Cases	Control	P
Hap1	GAGTGCACACTTAATGCATTAGCAGGTTAG	23.62	63.16 (0.23)	81.72 (0.27)	0.778
Hap2	GAGTGCACACTTAATGCATTAGCAGGTAAA	13.79	28.51 (0.11)	56.71 (0.18)	0.019*
Hap3	GAGTGCACACTTAATGCATTAGCAGGT ATG	3.10	7.64 (0.03)	6.00 (0.02)	-
Hap4	GAGTGCACACTTAATGCATCGACAGACTAG	3.79	20.25 (0.08)	2.28 (0.01)	0.006**
Hap5	GAGTGCACACTTAATGCATCGACAGATAAA	3.45	7.40 (0.03)	10.00 (0.03)	0.861
Hap6	GAGTGCACACTTAATGCATCGACAGATAAG	6.21	6.60 (0.02)	28.00 (0.09)	0.002**
Hap7	TATCACACGTGGTCCCAATTAGCAGGTAAG	2.76	10.01 (0.04)	8.11 (0.03)	0.346
Hap8	TATCACACGTGGTCCCAATCGACAGACAAA	2.41	10.81 (0.04)	4.00 (0.02)	0.024*
Hap9	GTGCGTGTACTTAACGCATTAGCAGGTAAG	2.07	3.03 (0.01)	8.00 (0.03)	0.146
Hap10	GAGTGCACACTTAATGCATTGACAGATAAG	4.48	6.62 (0.02)	16.00 (0.05)	0.132
Hap11	GAGTGCACACTTAATGCATTAGCAGGTATG	3.97	5.90 (0.02)	5.22 (0.012)	-
Hap12	GAGTGCACACTTAATGCATCGACAGATAAG	2.07	0	7.98 (0.03)	-
Hap13	TAGCGTGTGCTTACCGAATTAGCAGGTAAA	3.10	2.23 (0.01)	8.82 (0.03)	-
Hap14	TATCACACGTGGTCCCAATCGACAGACAAG	2.41	6.39 (0.03)	3.89 (0.02)	-
Others		22.77			

*Significant at 0.05 level. **Significant at 0.01 level.

DISCUSSION

The *SLA* genes play important roles in immune responses to both antibodies and cellular responses to conventional antigens, pathogenic viruses, and pathogens (Vaiman et al.,

1998; Zhou et al., 2005). The *DQA* genes are important production trait genes and are closely related to disease resistance in swine (Hickford et al., 2007; Janova et al., 2009). Extensive studies have examined the MHC II *DQA* genes in various species (Kamath and Getz, 2011), such as swine, cattle, and sheep. Liu et al. (2003) found 4 novel mutational sites and 2 novel alleles, *DQA-SLT 26* and *DQA-TC 21-1*, in *DQA* exon 2 of Swedish Landrace. There were 6 in 13 conserved regions, which differed from the published *DQA2* sheep sequences (Zhou et al., 2005); 37 ovine *DQA* haplotypes were detected in 520 sheep (Hickford et al., 2007). Janova et al. (2009) had reported that 13 new alleles were identified in the genus *Equus*. Le et al. (2012) identified 7 *SLA-DQA* alleles with 49 genotypes using genomic DNA-based high-resolution genotyping methods. One study reported greater *SLA* allelic diversity than had ever been observed in vertebrates (Kamath and Getz, 2011). Some studies demonstrated that the PCR-RFLP technique was not useful for polymorphism studies of the *SLA* genes because of its highly restrictive enzyme specificity (Zare, 2010). Yang et al. (2012, 2013) used PCR-SSCP technology to detect polymorphisms in *DRA* and *DQA* in the Large White, Landrace, and Duroc breeds. Therefore, the PCR-SSCP technique and DNA sequencing are considered to be efficient methods for comparison.

In this study, exon 2 contained 20 SNPs in the swine coding sequence and 10 amino acid variations, which was less than the previously reported 23 SNPs (Liu et al., 2003); this may occur because different breeds were used. *SLA-DQA* exons contained abundant polymorphism, and a total of 32 SNPs were detected in the 3 exons; however, the distribution of polymorphic sites was non-uniform. Compared to exon 4, exon 2 contained a larger number of SNPs. Compared to exons 2 and 3, exon 4 was relatively conserved. These conserved regions may be important functional gene areas. The cDNA codes for 255 amino acids containing 232 mature peptide amino acids in the *DQ* α chain and 23 signal peptide amino acids. In the *DQ* α chain, the Cys residues at positions 110 and 166 formed disulfide bonds, while positions 82 and 121 were glycosylation sites (Chardon et al., 1999). The position 82 amino acids correspond to the nucleotides 4134-4145 bp in exon 2 and were glycosylation sites, which may be related to gene conservation. Gene conservation is an animal self-protection mechanism and plays very important roles in immune regulation during the growth process. Additional analyses of other exon regions may reveal further genetic variation, which should be examined in future studies. Table 2 shows that exon 2 displayed an intermediate level of polymorphism, while exons 3 and 4 showed high polymorphism, which agreed with the results of Lunney et al. (2009) and Yang et al. (2013). Genetic diversity is essential for the preservation of adaptive potential of species and improvement of production potential of highly selected breeds.

Previously reports confirmed that the *SLA* gene was related to the death of post-weaning piglets, resistance to certain parasites, antigen recognition, immune responses, and withstanding exogenous pathogen infection (Tissot et al., 1989; Rothschild and Rubinsdy, 1998; Chen et al., 2005). The mortality rates before weaning caused by diarrhea were related to the *SLA* haplotypes (Renard and Vaiman, 1989; Yang et al., 2013). The diarrhea score of *DQA* exon 2 genotype CC (0.36 ± 0.19) was significantly lower than that of other genotypes (Yang et al., 2013). In this study, we did not detect this genotype in exon 2; however, genotype BB (0.42 ± 0.37) showed the lowest piglet diarrhea scores compared to others. Notably, genotype CC (0.36 ± 0.19) in Yang (2013) and genotype BB (0.42 ± 0.37) were both the most highly mutated genotypes in exon 2, with a total of 11 coincidental SNPs (CC:11/15, BB:11/14). This may be because of the difference in breeds and areas, generating significantly different

genotypes in natural selective processing. Individuals with higher diarrhea scores were more susceptible to diarrhea compared to individuals with lower diarrhea scores. During breeding, individuals with high diarrhea scores should be eliminated to improve disease resistance in population production.

Previous studies have reported strong LD among MHC genes, and the MHC class II region shows haplotype linkage in cow, human, and mouse, among others (Andersson et al., 1988; Andersson and Rask, 1988; Carrington, 1999; Yauk et al., 2003). In this study, we found that the haplotypes of *DQA* and *DRA* genes were in complete LD (Yang et al., 2014). Haplotypes were statistically analyzed for *SLA-DQA* "exon 2-exon 3-exon 4," and a total of 14 haplotypes were identified and found to be significantly lower than the theoretical value (4 x 6 x 4 = 96), indicating that the *SLA-DQA* exons were in genetic LD and that the haplotype distribution regarding the frequencies of haplotypes were significantly different (Table 5). In Table 5, the P values of Hap2, Hap4, Hap6, Hap8, and Hap10 between the cases and controls were significantly different, and association analysis suggested that Hap4 and Hap8 may contain diarrhea disease genes. The results reflected the interaction between multiple single-nucleotide variations, which was accordance with the hypothesis that inheritance of the haplotype combinations was more effective than that of 1 single-nucleotide variations (Fallin et al., 2001). The selection of MHC multiple haplotype genes may improve the accuracy of disease-resistant breeding.

In conclusion, this study was the first to report 3 novel SNPs in the *SLA-DQA* gene and described a significant association between polymorphism and piglet diarrhea in exons 2, 3, and 4. The results showed that the *SLA-DQA* gene was an informative genetic marker for identifying allele and haplotype linkages and variations within different populations. This study provides a foundation for marker-assisted selection in pig disease resistance breeding. In the future, it is essential that studies of the complex role of genetic variants in *SLA* genes and the mRNA expression levels in body tissue determine candidate genes for identifying biologically relevant associations between genotype and phenotype.

ACKNOWLEDGMENTS

Research supported by the Bio-Technology Special Program of Agriculture and Animal Husbandry of Gansu Province, China (Grant #GNSW-2008-04, #092NKDA036, and #0804NKCA065). The authors would like to thank the College of Animal Science and Technology, Gansu Agricultural University for providing a laboratory and for comments and suggestions.

REFERENCES

Andersson L and Rask L (1988). Characterization of the MHC class II region in cattle. The number of DQ genes varies between haplotypes. *Immunogenetics* 27: 110-120.

Andersson L, Lundén A, Sigurdardottir S, Davies CJ, et al. (1988). Linkage relationships in the bovine MHC region. High recombination frequency between class II subregions. *Immunogenetics* 27: 273-280.

Bao WB, Ye L, Chen Z, Liu L, et al. (2012). Relationship between the expression level of SLA-DQA and *Escherichia coli* F18 infection in piglets. *Gene* 494: 140-144.

Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Carrington M (1999). Recombination within the human MHC. *Immunol. Rev.* 167: 245-256.

Chardon P, Renard C and Vaiman M (1999). The major histocompatibility complex in swine. *Immunol. Rev.* 167: 179-192.
Chen FX, Xie J, Li NL, Zhou Y, et al. (2005). Novel SLA-DQ alleles and their recombinant molecules in xenogeneic stimulation of human T cells. *Transpl. Immunol.* 14: 83-89.
Cooper VL and Helman RG (2000). Diagnosis of neonatal pig diarrhea. *Vet. Clin. North Am. Food. Anim. Pract*. 16: 117-133.
Escayg AP, Hickford JGH, Montgomery GW, Dodds KG, et al. (1996). Polymorphism at the ovine major histocompatibility complex class II loci. *Anim. Genet.* 27: 305-312.
Eveline MIA, Patrick K, Aloysius EI and Zhao X (2008). A critical analysis of disease-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. *Mamm. Genome* 19: 226-245.
Fallin D, Cohen A, Essioux L, Chumakov I, et al. (2001). Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res.* 11: 143-151.
Gautschi C and Gaillard C (1990). Influence of major histocompatibility complex on reproduction and production traits in swine. *Anim. Genet.* 21: 161-170.
Hickford JGH, Zhou H and Fang Q (2007). Haplotype analysis of the DQA genes in sheep: evidence supporting recombination between the loci. *J. Anim. Sci.* 85: 577-582.
Ho CS, Lunney JK, Lee JH, Franzo-Romain MH, et al. (2010). Molecular characterization of swine leukocyte antigen class II genes in outbred pig populations. *Anim. Genet.* 41: 428-432.
Janova E, Matiasovic J, Vahala J, Vodicka R, Dyke V, et al. (2009). Polymorphism and selection in the major histocompatibility complex DRA and DQA genes in the family Equidae. *Immunogenetics* 61: 513-527.
Kamath PL and Getz WM (2011). Adaptive molecular evolution of the Major Histocompatibility Complex genes, DRA and DQA, in the genus *Equus*. *BMC Evol. Biol.* 11: 128-145.
Kelly DJ, O'Brien JJ and McCracken KJ (1990). Effect of creep feeding on the incidence, duration and severity of post-weaning diarrhoea in pigs. *Res. Vet. Sci.* 49: 223-228.
Kelley J, Walter L and Trowsdale J (2005). Comparative genomics of major histocompatibility complexes. *Immunogenetics* 56: 683-695.
Lechler R, Batchelor R and Lombardi G (1991). The relationship between MHC restricted and allospecific T cell recognition. *Immunol. Lett.* 29: 41-50.
Le MT, Choi H, Choi MK, Nguyen DT, et al. (2012). Comprehensive and high-resolution typing of swine leukocyte antigen DQA from genomic DNA and determination of 25 new SLA class II haplotypes. *Tissue Antigens* 80: 528-535.
Liu B, Zhu ZM, Yu M, Cao JH, et al. (2003). Characterization of novel alleles and new mutation sites of porcine MHC class II DQA. *Yi Chuan Xue Bao* 30: 955-960.
Lunney JK, Ho CS, Wysocki M and Smith DM (2009). Molecular genetics of the swine major histocompatibility complex, the SLA complex. *Dev. Comp. Immunol*. 33: 362-374.
Maddox-Hyttel C, Langkjær RB, Enemark HL and Vigre H (2006). *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs-occurrence and management associated risk factors. *Vet. Parasitol.* 10: 48-59.
Mallard BA, Kennedy BW and Wilkie BN (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.
Nagy B and Fekete PZ (1999). Enterotoxigenic *Escherichia coli* (ETEC) in farm animal. *Vet. Res.* 30: 259-284.
Patch JR, Pedersen LE, Toka FN, Moraes M, et al. (2011). Induction of foot-and-mouth disease virus-specific cytotoxic T cell killing by vaccination. *Clin. Vaccine Immunol*. 18: 280-288.
Racioppi L, Ronchese F, Schwartz RH and Germain RN (1991). The molecular basis of class II MHC allelic control of T cell responses. *J. Immunol.* 147: 3718-3727.
Renard C and Vaiman M (1989). Possible relationships between SLA and porcine reproduction. *Reprod. Nutr. Dev.* 29: 569-576.
Renard C, Hart E, Sehra H, Beasley H, et al. (2006). The genomic sequence and analysis of the swine major histocompatibility complex. *Genomics* 88: 96-110.
Rothschild MF and Rubinsdy A (1998). The Genetics of the Pig. CAB International, Wallingford, 163-166.
Sambrook J and Russell DW (2001). Molecular cloning: A Laboratory Manual. 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Sanguinetti CJ, Dias Neto E and Simpson AJ (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 914-921.
Stephens M and Donnelly P (2003). A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet*. 73: 1162-1169.
Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731-2739.
Takeshima SN, Sarai Y and Saitou N (2009). MHC class II DR classification based on antigen-binding groove natural

selection. *Biochem. Biophys. Res. Commun.* 385: 137-142.
Tissot RG, Beattie CW and Amoss MS Jr (1989). The swine leucocyte antigen (SLA) complex and Sinclair swine cutaneous malignant melanoma. *Anim. Genet.* 20: 51-57.
Vaiman D, Mecier D, Moazami-Goudarzi K, Eggen A, et al. (1994). A set of 99 cattle microsatellites: characterization, synteny mapping, and polymorphism. *Mamm. Genome* 5: 288-297.
Vaiman M, Chardon P and Rothschild MF (1998). Porcine major histocompatibility complex. *Rev. Sci. Tech*. 17: 95-107.
Vander PJJ, Groenen MAM and Dijkho RJM (1990). The nucleotide sequence of the bovine MHC class II alpha genes: DRA, DQA and DYA. *Immunogenetics* 31: 29-36.
Wei LH (2004). In: Methods in Molecular Biology (Spencer JFT and de Spencer AL R, eds.). Microflora of the gastrointestinal tract: A review. Humana Press, New York, 491-502.
Yang QL, Kong JJ, Zhao SG, Liu LX, et al. (2012). Polymorphism of SLA-DRA exon 2 gene and its correlation with piglet diarrhea. *Acta Vet. Zootech. Sin.* 43: 1020-1027.
Yang QL, Kong JJ, Wang DW, Zhao SG, et al. (2013). Swine leukocyte antigen-DQA gene variation and its association with piglet diarrhea in Large White, Landrace and Duroc. *Asian Australas. Asian Australas. J. Anim. Sci*. 26: 1065-1071.
Yang QL, Zhao SG, Wang DW, Feng Y, et al. (2014). Association between genetic polymorphism in the swine leukocyte antigen-DRA gene and piglet diarrhea in three Chinese pig breeds. *Asian Australas. J. Anim. Sci*. 27: 1228-1235.
Yauk CL, Bois PR and Jeffrey AJ (2003). High-resolution sperm typing of meiotic recombination in the mouse MHC beta gene. *EMBO J.* 22: 1389-1397.
Zare N (2010). Evaluation of polymorphism in BMPR-1B gene associated with litter size in Ghezel sheep using PCR-RFLP. The 4th Congress on Animal Science, Tehran University, Tehran.
Zhou HJ, Hickford GH and Fang Q (2005). Polymorphism of the DQA2 gene in goats. *J. Anim. Sci.* 83: 963-968.