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Tissue Distribution, SNP Detection and Association Study with Immune Traits of Porcine *LBP* and *CD14* Genes*

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ABSTRACT : Lipopolysaccharide binding protein (LBP) and CD14 protein play important roles in the defense against infection of Gram-negative bacteria. In the present study, tissue distribution and polymorphism of porcine *LBP* and *CD14* genes were analyzed. Real-time PCR results showed that the porcine *LBP* gene was especially highly expressed in liver, while *CD14* gene was highly expressed in liver and spleen tissues. A 1,732 bp cDNA fragment of porcine *LBP* gene and a 1,682 bp genomic DNA fragment of *CD14* gene were isolated. Polymorphisms were identified in these two fragments and showed that there were 14 potential SNPs in the porcine *LBP* gene and 3 potential SNPs in the porcine *CD14* gene. Three SNPs, 292G/A (Gly/Ser), 1168G/A (Ala/Thr) of the *LBP* gene and -61G/A of the *CD14* gene, were genotyped using restriction fragment length polymorphism (RFLP) method. Association analyses indicated that polymorphism of the 292G/A locus was significantly associated with porcine immune traits hematocrit (HCT), IgG and delayed-type hypersensitivity (DTH) ($p<0.01$), and the 1168G/A locus was significantly associated with HCT and mean corpuscular volume (MCV) traits ($p<0.05$). No significant association was found between the -61G/A locus and immune traits of the pig. Our data indicated that the *LBP* gene was significantly associated with immune traits of pig. Also, we identified some SNPs which may be useful markers for disease-resistant breeding of pigs. (**Key Words :** Pig, LBP, CD14, TLR4, SNP, Immune Trait)

INTRODUCTION

Toll like receptor (TLR) signal transduction participates in the activation of the innate immune system. This signal transduction pathway is initiated by the recognition of structural components of microorganisms (Medzhitov et al., 1997). In mammalian systems, 11 members of TLR (TLR1-11) have been characterized (Medzhitov et al., 1997; Rock et al., 1998; Palladino et al., 2007). TLR4 signal plays an important role in defense against infection of Gram-negative bacteria (Takeuchi et al., 1999; Palsson-McDermott and O'Neill, 2004). Lipopolysaccharide (LPS, also termed as endotoxin) is the major component of the outer membrane of Gram-negative bacteria and the LPS recognition initiates TLR4 signal transduction (Heumann and Roger, 2002; Palsson-McDermott and O'Neill, 2004). LBP and CD14 are two key proteins for LPS recognition (Haziot et al., 1997; Jack et al., 1997). LBP is a 60 kDa glycoprotein found in serum, which binds LPS and forms LPS-LBP complex, and transfers LPS from bacterial membranes to CD14 (Schumann et al., 1990). CD14, a 55 kDa glycoprotein, exists as a glycosylphosphatidylinositol-anchored form on the surfaces of myeloid lineage cells (mCD14) and a soluble CD14 form in serum (sCD14) (Frey et al., 1992; Pugin et al., 1993). CD14 forms a stable complex with complex LPS-LBP and presents LPS to the TLR4 to activate the TLR4 signaling pathway (Ulevitch and Tobias, 1999; Beutler, 2000). The immune reaction induced by TLR4 signal was associated with the release of cytokine including tumor necrosis factor alpha (TNF-a), IL-1, IL-6, IL-8 and IFN (Beutler, 2000; Kawai et al., 2001). The activation of the immune response results in depression and clearance of the infection. Therefore, the recognition of LPS is very important for the activation of the TLR4 signaling

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Table 1. Primers used for porcine *LBP* and *CD14* gene isolation, SNPs detection and mRNA distribution analysis

Gene	Primers	5'-3' sequence	Length (bp)	TM (°C)
LBP	cloning	CTGCATCGGACCTGACCCA	1,732	61
		TGCCCTTCAAGAAAGTACCAGC		
	292G/A	GAACCACCTGTCTGCCTGAAG	287	63
		CGGGAGCAGAGTCAACTTACAG		
	1168G/A	TCACTGAGATGTCTGTTTCGAG	164	61
		CTGTAGGAATCCAGTGATCTTGC		
	Q_PCR	CTCTGACCTACAGCCTTACCTC	312	64
		GGTGGAACCAAGTCATCTGTG		
CD14	cloning	CTGAGGCACTCGGGATCTTC	1,682	61
		TGGTTGGTTGAGTTGTCCTGAG		
	-61G/A	GGTGGGAGGTGGCAGAGTTCA	166	62
		AACAAATAGCTCTCGAAAGGGCTGTGGATC		
	Q_PCR	GGTGGGAGGTGGCAGAGTTCA	209	64
		GCAAGCGCACCATGGTCGAT		
	β-actin	GGACTTCGAGCAGGAGATGG	233	64
		GCACCGTGTTGGCGTAGAGG		

pathway and resistance against infection. The genetic variation in the ability to recognize pathogens may be related to the risk of infection. Previous studies indicated that polymorphism of the *CD14* gene was highly associated with infectious disease. A polymorphism at the promoter of the *CD14* gene (-159T/C) in humans was significantly associated with infection of brucellosis, chronic hepatitis B, asthma and allergic diseases (Buckova et al., 2006; Haidari et al., 2006; Keskin et al., 2006; Mohammad Alizadeh et al., 2006). Polymorphisms of the *LBP* gene (98Cys/Gly; 436Pro/Leu) were associated with sepsis (Hubacek et al., 2001). Thus *LBP* and *CD14* are two important genes associated with immunity and disease infection.

The objective of this research was to isolate porcine *LBP* and *CD14* genes, to analyze their tissue distribution and to screen their polymorphisms. Association analysis results indicated that *LBP* gene was closely related to some immune traits of the pig, which may be considered as a candidate gene for immune capacity.

MATERIALS AND METHODS

Isolation of porcine *LBP* and *CD14* genes

For porcine *LBP* isolation, human *LBP* mRNA sequences (GenBank accession number NM_004139) were compared to all sequences available in the pig EST databases using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST). The porcine ESTs that shared more than 80% sequence identity to the corresponding human cDNA were selected and assembled using the DNAStar program (Madison, WI, USA). To confirm the cDNA sequence of porcine *LBP* gene, RT-PCR was performed using the specific primers (Table 1). For *CD14* genome isolation, porcine *CD14* mRNA sequence (GenBank accession number DQ357211) was used to search DNA fragments in the pig genome database. Then, primers were designed to isolate the genome of porcine *CD14* gene. The PCR profiles were 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature, 60 s at 72°C and a final extension of 5 min at 72°C. All the PCR products were purified and subsequently cloned into the pEGM-T-Easy vector (Promega, USA) prior to sequencing.

Real-time PCR analysis of gene expression patterns

The Wuzhishan pig is one of the famous Chinese indigenous mini-pigs (Wang et al., 2007). Eleven tissues, including heart, liver, spleen, lung, kidney, fat, muscle, lymph node, small intestine, large intestine and brain, were obtained from four 18- to 24-month-old Wuzhishan mini-pigs for the mRNA tissue distribution analysis. Total RNA was extracted from each sample using Trizol reagent (Invitrogen, San Diego, CA) and then treated with RNase-free DNase I (MBI Fermentas, St. Leon-Rot, Germany). RNA concentration was determined, and the same amount of RNA from each targeted tissue sample of different individuals was mixed to form the RNA pool. The pooled RNA of each sample was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Promega, USA) and oligo (dT) primers. Real-time PCR was performed in a 20 μl mixture containing 1×PCR buffer (TaKaRa, Dalian, China), 3.0 mM $MgCl_2$, 100 μM each dNTP, 0.3 μM gene-specific primers (Table 1), 0.3×SYBR Green I , 2 U Taq DNA polymerase (TaKaRa, Dalian, China), and 2 μl template cDNA. Reactions were carried out in an Opticon 2 real-time cycler (MJ Research, Waltham, MA), and the cycling conditions consisted of an initial 5 min at 95°C followed by 35 cycles of 15 s at 95°C (for denaturation), 30 s at 64°C (for annealing), 30 s at 72°C (for polymerization) and fluorescence acquisition at 83°C for 1 s. The standard curve was generated from a series of diluted plasmid containing the targeted gene fragment. The PCR efficiency

of each specific gene was calculated according to its standard curve. PCR reactions were performed in triplicate, and gene expression levels were quantified relative to the expression of *β-actin* using Gene Expression Macro software (Bio-Rad, Richmond, CA) employing an optimized comparative Ct (ΔΔCt) value method. The expression was considered undetectable when the Ct value of the targeted gene exceeded 35.

SNP identification and association analysis

Potential polymorphism sites were analyzed by sequence comparisons using DNAstar software (DNAstar Inc., Madison, WI, USA). cDNA samples from three Wuzhishan and three Landrace pigs were used as PCR template for *LBP* isolation and DNA samples used for *CD14* isolation. PCR products were sequenced by a commercial service. All the sequence information of these two genes, including our PCR results, the ESTs and genome DNA information available on NCBI, were collected to analysis their potential SNPs. The locus, when different alleles appeared more than two times, was considered as one potential SNP site. Additional primers were designed to confirm the potential SNPs by PCR and sequence. Genetic variation was analyzed in five unrelated pig breeds, which were Tongcheng, Wuzhishan, Laiwu, Yorkshire and Landrace.

Association analysis was performed in the experimental group (n = 149). This group consists of three pure-blood populations including Tongcheng (T, n = 41), Landrace (L, n = 13) and Yorkshire (Y, n = 18), and two crossbred populations including LYT (L♂×YT♀, n = 39) and YLT (Y♂×LT♀, n = 38). All the pigs were fed and managed in the same way. Blood of 20-wk-old pigs was collected to determine the basic immune traits, RBC, HCT, MCV and red blood cell distribution width (RDW), which were detected using a blood cell autoanalyzer (MEK-5216K). The concentration of IgG was determined by the radial immunodiffusion method. The delayed –type hypersensitivity (DHT) trait was detected using phytohemagglutinin (PHA) skin test according to the method of van Heugten with a minor modification (van Heugten et al., 1994). Generally, two-month-old pigs were injected with PHA at 0.3 ml (150 μg/ml) in skin of the left oxter and 24 h later, values of the diameter and transverse diameter of the red maculation were detected. The mean value of the diameter and transverse diameter is the value of the DTH trait which was used to detect *in vivo* cellular immunity. Detailed procedures were described previously (Liu et al., 2003).

The general linear model (GLM) was used to estimate the association between the genotypes of each gene and the immune traits by using SPSS software, version 11.5. According to the character of the population, the model used for trait association analysis is described as follows:

$$Y_{ijkl} = \mu+P_i+G_j+B_k+(PG)_{ij}+(PB)_{ik}+(GB)_{jk}+e_{ijkl},$$

Where, Y_{ijkl} = l^{th} trait measured on animal; μ = overall mean; P_i = fixed effect of the i^{th} population (i = 1, 2, 3, 4, 5); G_j = fixed effect of j^{th} genotype (j = 1, 2, 3); B_k = fixed effect of k^{th} batch (phenotypic data were recorded in two periods, k = 1, 2); $(PG)_{ij}$ = effect of interaction i^{th} population×j^{th} genotype; $(PB)_{ik}$ = effect of interaction i^{th} population×k^{th} batch; $(GB)_{jk}$ = effect of interaction j^{th} genotype×k^{th} batch; e_{ijkl} = error term.

RESULTS

Isolation of porcine *LBP* and *CD14* genes

The cDNA fragment of the porcine *LBP* gene consisted of 1732 nucleotides (nt) containing an ORF of 1446 nt, encoding a protein of 481 amino acid residues. The presumptive protein of porcine *LBP* contained two conserved consensus domains BPI 1 (cd00025.5) and BPI 2 (cd00026.5) compared with human LBP. The 5'-untranslated region (5'-UTR) was 37 nt and the 3'-untranslated region (3'-UTR) was 249 nt. Porcine *CD14* genomic DNA was 1,682 bp, which contained an ORF of 1,122 bp, an intron of 80 bp, 76 bp of 3' UTR and 404 bp of the promoter region. The intron was downstream of the translated start codon ATG.

Tissue expression patterns of porcine *LBP* and *CD14* genes

Q-PCR analysis was performed to determine the relative mRNA expression of both *LBP* and *CD14* in various pig tissues; the housekeeping gene, *β-actin*, was used for endogenous control. Porcine *LBP* mRNA was most abundant in liver, and weak expressions were detected in heart, spleen, small intestine, brain and kidney. Porcine *CD14* mRNA was expressed at the highest level in spleen, followed by liver, modestly expressed at kidney, lymph node, fat and large intestine, and weakly expressed in small intestine, muscle, lung and brain (Figure 1).

Polymorphism detection and association analysis

The polymorphisms of *LBP* cDNA and *CD14* DNA fragments were detected by sequence comparisons using DNAstar software (Madison, WI, USA). According to our results, the 1,732 bp cDNA fragment of *LBP* gene contained 14 potential SNPs which were -16T/C, -2T/G, 51T/C, 166G/A (Glu/Lys), 292G/A (Gly/Ser), 440G/A (Gly/Glu), 465C/A, 495T/C, 504A/G, 1168G/A (Ala/Thr), 1215A/G, 1225A/G (Val/Met), 1377T/C and 1607G/A. The 1,682 bp DNA fragment of *CD14* gene contained 3 potential SNPs which were -61G/A, 587A/G and 1246A/G. The nucleotide

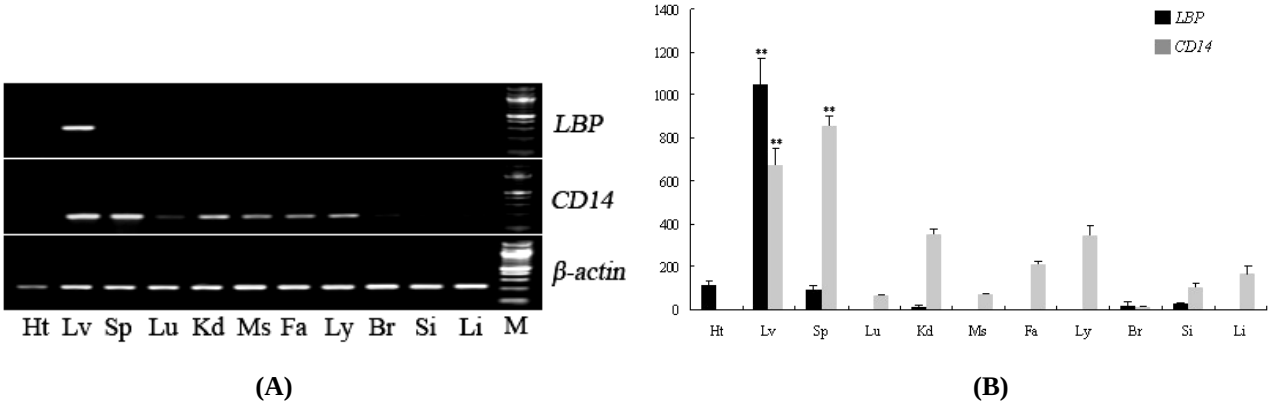

Figure 1. The tissue expression patterns of the porcine *LBP* and *CD14* genes. (A) Gel photos of PCR results of *LBP*, *CD14* and *β-actin* genes. (B) The real-time PCR results, the error bars represent the SD (n = 3). Ht: heart, Lv: liver, Sp: spleen, Lu: lung, Kd: kidney, Ms: muscle, Fa: fat, Ly: lymph knot, Br: brain, Si: small intestine, Li: large intestine. ** Means that the expression of *CD14* in liver is significantly higher than that in other tissues. Similarly, *LBP* in liver and spleen tissues is relatively higher.

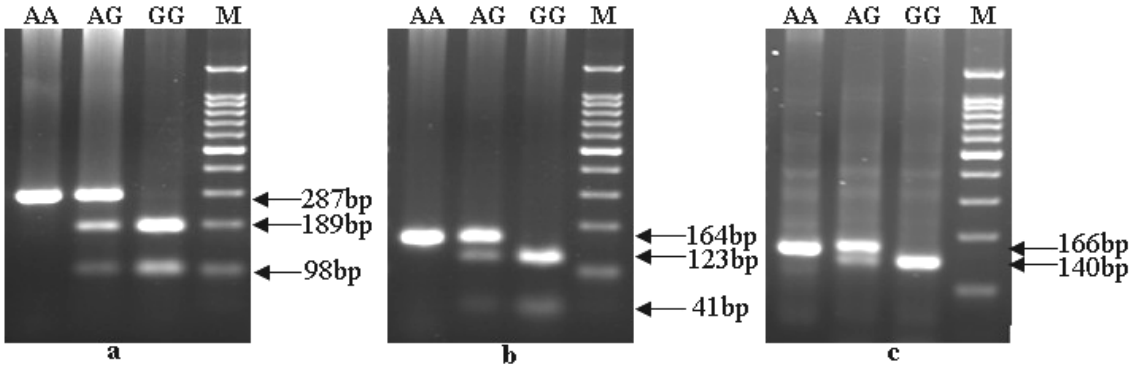

Figure 2. RFLP analysis of porcine *LBP* and *CD14* genes polymorphisms. Two polymorphisms 292G/A and 1168G/A of *LBP* gene were detected by *Msp I* (AA 287 bp, GG 189/98 bp, AG 287/189/98 bp) and *Bsh1236 I* (AA 164 bp, GG 123/41 bp, AG 164/123/41 bp), respectively (Figure 2a, 2b); the SNP -61G/A of *CD14* gene was detected by digestion with *BamH I* (AA 166 bp, GG 140/26 bp, AG 166/140/26 bp) (Figure 2c). M: DNA ladder.

a of translation start codon ATG was set at 1 in this study. Three SNPs, 292G/A (Gly/Ser) and 1168G/A (Ala/Thr) of *LBP* gene and -61G/A of *CD14* gene, were confirmed using the RFLP method. The 292G/A (Gly/Ser) of *LBP* was detectable by digestion with *Msp I* (AA 287 bp, GG 189/98 bp, AG 287/189/98 bp) (Figure 2a). Allele frequency analysis of this SNP revealed a much higher frequency of allele G in three Chinese indigenous breeds, whereas allele A had higher frequency in Landrace and Large White breeds (Table 2) The 1168G/A (Ala/Thr) of *LBP* was detectable by digestion with *Bsh1236 I* (AA 164 bp, GG 123/41 bp, AG 164/123/41 bp) (Figure 2b), and the allele frequency analysis revealed a much higher frequency of allele G in Chinese indigenous breeds than foreign breeds. The -61G/A of *CD14* was detectable by digestion with *BamH I* (AA 166 bp, GG 140/26 bp, AG 166/140/26 bp) (Figure 2C). Allele frequency analysis indicated that allele A had higher frequency in Chinese indigenous breeds than

Table 2. Genotypes and allelic frequencies for the 292G/A, 1168G/A and -61G/A polymorphisms in several pig breeds

Breeds	292G/A/*Msp I* (n = 159)					1168G/A/ *Bsh1236 I* (n = 168)					-61G/A/*BamH I* (n = 164)				
	Genotypes			Frequency		Genotypes			Frequency		Genotypes			Frequency	
	AA	AG	GG	A	G	AA	AG	GG	A	G	AA	AG	GG	A	G
Wuzhishan	0	3	31	0.04	0.96	4	15	19	0.3	0.7	40	0	0	1	0
Tongcheng	2	0	39	0.05	0.95	6	17	15	0.38	0.62	35	2	1	0.95	0.05
Laiwu	0	2	30	0.03	0.97	0	2	35	0.03	0.97	8	18	10	0.47	0.53
Landrace	23	3	0	0.94	0.06	21	6	0	0.89	0.11	4	9	10	0.37	0.63
Yorkshire	23	2	1	0.92	0.08	26	2	0	0.96	0.04	3	8	16	0.26	0.74

Table 3. Association analysis of the *LBP* SNP 292G/A (Gly/Ser) with RBC, HCT, IgG, DTH and RDW traits

Genotypes	N	RBC	HCT	IgG	DTH	RDW
AA	64	6.27±0.21	36.59±1.27[a]	44.50±2.31[a]	9.04±0.25[ac]	18.37±0.31
AG	42	6.35±0.21	36.22±1.25[a]	44.18±2.24[a]	8.55±0.25[a]	18.64±0.31
GG	39	6.84±0.17	41.04±1.00[c]	53.79±1.79[c]	9.60±0.20[c]	18.75±0.24
p-value*		0.059	0.003	<0.001	0.004	0.623

[a, b; b, c] Means a significant difference between different genotypes ($p<0.05$); [a, c] means $p<0.01$.

* Means the probability of F-test for the genotype effect. The phenotypic value = mean±SE.

Table 4. Association analysis of the *LBP* SNP 1168G/A (Ala/Thr) with the RBC, HCT, MCV, IgG, and DTH traits

Genotypes	N	RBC	HCT	MCV	IgG	DTH
AA	73	6.30±0.16	36.02±0.94[a]	57.55±0.53	45.94±1.68	9.19±0.19
AG	54	6.51±0.17	37.78±1.00[ac]	58.13±0.57	48.81±1.80	8.90±0.20
GG	15	6.85±0.27	41.04±1.59[c]	59.96±0.90	53.67±2.85	9.48±0.32
p-value*		0.193	0.026	0.072	0.066	0.277

[a, b; b, c] Means a significant difference between different genotypes ($p<0.05$); [a, c] Means $p<0.01$.

* Means the probability of F-test for the genotype effect. The phenotypic value = mean±SE.

in foreign breeds (Table 2).

Trait association analysis was performed in our experimental group. Among the immune traits studied, there was no significant interaction effects between population and genotype (P×G), between population and batch (P×B) and between genotype and batch (G×B) ($p>0.05$). The genotype and immune traits association results showed that 292G/A of *LBP* gene was significantly associated with HCT ($p<0.01$), IgG ($p<0.01$) and DTH ($p<0.01$) traits, and the individuals with genotype GG had significantly higher values of HCT, IgG and DTH than those individuals with genotype AA or AG (Table 3). The 1168G/A of *LBP* gene was significantly associated with HCT ($p<0.05$) traits. HCT of the individuals with GG genotype was significantly higher than that of individuals with genotype AA or AG (Table 4). There was no significant association between the polymorphism of *CD14* gene and the immune traits of the pig (Table 5).

DISCUSSION

Toll like receptor 4 signal plays an important role in resisting the infection of Gram-negative bacteria. LBP and CD14 are two key components for initiation of TLR4 signal. Thus, these two genes may be associated with immune capacity of the pig. In this study, the porcine *LBP* and *CD14* genes were cloned. The mRNA expression patterns of these two genes were analyzed and the polymorphisms were detected. Also, genetic variation and traits association were studied.

The *LBP* and *CD14* mRNA expression patterns in different porcine tissues were analyzed by real-time PCR. The results showed that porcine *LBP* mRNA was highly abundant in liver tissue, and *CD14* mRNA was highly expressed at spleen and liver tissues. Microarray analyses showed that both human *LBP* and *CD14* genes were abundantly expressed in liver tissue (http://genome.ucsc.edu/). Thus, the tissue distributions of porcine *LBP* and *CD14* mRNA were similar to those of human. Moreover, CD14 was highly expressed on the surface of monocytes/macrophages and strongly up-regulated during the differentiation of monocytic precursor cells into monocytes. At present, CD14 has been used as a differentiation marker for monocytic cell lineage (Griffin et al., 1981; Goyert et al., 1988; Simmons et al., 1989) . Larger amounts of monocytic cells exist in spleen, which may be the reason that porcine *CD14* mRNA was abundant in spleen tissue. In the rat, both *LBP* and *CD14* mRNA levels increased about 20 fold in hepatocytes when treated with LPS (Wan et al., 1995; Liu et al., 1998). Thus, the hepatocyte was one of the important sources of the expression of *LBP* and *CD14* genes. Furthermore, most acute-phase proteins of infection were produced by liver (Janzen et al., 1987; Darlington et al., 1989; Streetz et al., 2001). Therefore, liver may be the important organ in defense against the infection of bacteria, including Gram-negative bacterial infection, through the

Table 5. Association Analysis of the *CD14* SNP -61G/A with the MCV, IgG, DTH and RDW traits

Genotypes	N	MCV	IgG	DTH	RDW
AA	51	58.02±0.85	49.18±2.46	9.50±0.28	18.44±0.36
AG	40	58.96±0.92	43.33±2.69	8.69±0.31	18.65±0.40
GG	50	57.01±1.11	46.21±3.19	8.92±0.36	18.51±0.47
p-value*		0.434	0.231	0.097	0.913

[a, b; b, c] Means a significant difference between different genotypes ($p<0.05$); [a, c] Means $p<0.01$.

* Means the probability of F-test for the genotype effect. The phenotypic value = mean±SE.

TLR4 signaling pathway.

In this study, polymorphisms of porcine *LBP* and *CD14* genes have been detected. The results showed that the 1,732 bp fragment of the *LBP* gene contains 14 potential SNPs and the 1,682 bp fragment of the *CD14* gene contains 3 potential SNPs. According to the results of the human genome sequence, each polymorphism occurred every 807 bp on average (Haga et al., 2002). The SNPs in *LBP* occurred almost 8 fold higher than the average level. Thus, *LBP* was rich in polymorphisms. Previous studies indicated that antigen interaction genes, such as MHC, were rich in SNPs (Klein et al., 1993; Hughes and Yeager, 1998). LBP is the binding protein of LPS and minor difference in LPS among bacteria may have caused *LBP* to become rich in SNPs during evolution. Two SNPs, 292G/A and 1168G/A, of *LBP* and one SNP, -61G/A, of *CD14* were further studied. 292G/A (Gly/Ser) and 1168G/A (Ala/Thr) were located at the conserved functional domains BPI 1 and BPI 2 of LBP, respectively. -61G/A was located at the promoter region of *CD14* gene. The results of transcription factor locus analyses indicated that polymorphism -61G/A was located at the locus of the T cell-specific transcription factor 1 (TCF-1) (http://www.cbil.upenn.edu/cgi-bin/tess/tess). TCF-1 locus (AAAAG) presents when the allele is A, whereas, TCF-1 locus is abolished when the allele is G. TCF-1 was widely expressed during embryogenesis and restricted to lymphocytes postnatally (Oosterwegel et al., 1993). TCF-1 plays important roles in cellular surface protein expression of lymphocytes (Waterman and Jones, 1990). In this study, porcine *CD14* was widely expressed in many tissues. Whether this polymorphism really impacted on the mRNA level of the porcine *CD14* gene needs further study. Genetic variation analyses showed that the allele frequency was different between Chinese indigenous breeds and foreign breeds. The G allele of polymorphisms 292G/A and 1168G/A of the *LBP* gene had a much higher proportion in Chinese indigenous breeds than in foreign breeds, whereas the contrary was true for the -61G/A of the *CD14* gene,in which the A allele was abundant in Chinese indigenous breeds and the G allele was abundant in foreign breeds. These results showed the genetic difference between Chinese indigenous and foreign breeds.

Previous studies indicated that SNPs of *LBP* and *CD14* were significantly associated with risk of infectious disease of humans (Hubacek et al., 2001; Buckova et al., 2006; Keskin et al., 2006; Mohammad Alizadeh et al., 2006). The -159T/C of *CD14* was associated with level of immune factor in humans, IgE level being higher in serum of humans with CC genotype than with CT and TT genotypes (Kang et al., 2006). Moreover, polymorphisms of genes related to the TLR4 signaling pathway were associated with immune response. For example, the polymorphism 896G/A of *TLR4* was significantly ($p = 0.027$) associated with an increased risk of severe sepsis (Barber et al., 2006); the *TNF-alpha* promoter polymorphism -308A/G might be associated with asthma or bronchial hyper-responsiveness (Hong et al., 2007). According to the human studies, we deduced that some polymorphisms of genes in the TLR4 signaling pathway were associated with infectious disease and immune levels. In this study, we detected SNPs of porcine *LBP* and *CD14* genes and trait association analysis was performed in our experimental group. Our data showed that there were some significant associations between the genotypes and immune traits investigated. 292G/A of the *LBP* gene was associated with traits HCT, IgG and DTH ($p<0.01$), 1168G/A of *LBP* gene was associated with trait HCT ($p<0.05$) and -61G/A of *CD14* gene was not associated with any immune traits studied. Therefore, the *LBP* gene may be associated with the immune capacity of pigs and the two SNPs of *LBP* studied here may be useful markers in animal disease-resistant breeding. Furthermore, the *CD14* gene may also play an important role in the immune system of the pig, though no significant association was found in this study. New SNPs of the *CD14* gene should be detected in future studies. In addition, the character of the experimental group, such as the number of samples, fixed effects and random effects, was very important for the accuracy of the association analysis (Lukovic et al., 2007). Therefore, our association results of genotype and immune traits should be further confirmed in other experimental groups.

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