GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Novel SNPs of the mannan-binding lectin 2 gene and their association with production traits in Chinese Holsteins

Z.L. Zhao[1,2], C.F. Wang[2], Q.L. Li[2], Z.H. Ju[2], J.M. Huang[2], J.B. Li[2], J.F. Zhong[2] and J.B. Zhang[1]

[1]College of Animal Husbandry and Veterinary, Jilin University, Changchun, P.R. China
[2]Centre of Dairy Cattle Research,
Shandong Academy of Agricultural Science, Jinan, P.R. China

Corresponding author: J.B. Zhang
E-mail: zhaozhongli954@sohu.com

Genet. Mol. Res. 11 (4): 3744-3754 (2012)
Received January 18, 2012
Accepted May 25, 2012
Published October 15, 2012
DOI http://dx.doi.org/10.4238/2012.October.15.6

ABSTRACT. The mannan-binding lectin gene (*MBL*) participates as an opsonin in the innate immune system of mammals, and single nucleotide polymorphisms (SNPs) in *MBL* cause various immune dysfunctions. In this study, we detected SNPs in *MBL2* at exon 1 using polymerase chain reaction single-strand conformation polymorphism analysis and DNA sequencing techniques in 825 Chinese Holstein cows. Four new SNPs with various allele frequencies were also found. The g.1164 G>A SNP was predicted to substitute arginine with glutamine at the N-terminus of the cysteine-rich domain. In the collagen-like domain, SNPs g.1197 C>A and g.1198 G>A changed proline to glutamine, whereas SNP g.1207 T>C was identified as a synonymous mutation. Correlation analysis showed that the g.1197 C>A marker was significantly correlated to somatic cell score (SCS), and the g.1164 G>A locus had significant effects on SCS, fat content, and protein content ($P < 0.05$), suggesting possible roles of these SNPs in the host response against mastitis. Nine haplotypes and nine

haplotype pairs corresponding to the loci of the 4 novel SNPs were found in Chinese Holsteins. Haplotype pairs MM, MN, and BQ were correlated with the lowest SCS; MN with the highest protein yield; MM with the highest protein rate, and MN with the highest 305-day milk yield. Thus, MM, MN, and BQ are possible candidates for marker-assisted selection in dairy cattle breeding programs.

Key words: Bovine; *MBL2*; SNPs; Somatic cell score

INTRODUCTION

Mastitis, an inflammatory disease of the mammary gland caused by intramammary infections, is a common and costly disease in dairy cattle. It is associated with reduced milk yield, degraded milk quality, reduced lactation persistence, and early culling (Seegers et al., 2003). Environmental and contagious pathogens including *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and *Staphylococcus aureus* are, by far, the main causes of mastitis (Chaneton et al., 2008). Therapeutic, prophylactic, and management strategies have been proposed to minimize the occurrence and effects of this complex disease. However, an approach based on improving host genetics through molecular marker selective breeding is becoming widely accepted. Gaining insight into the genetic variation of candidate genes and their association with milk production traits and somatic cell score (SCS), which presents a genetically positive correlation with clinical mastitis, is useful (Rupp and Boichard, 1999).

Mannan-binding lectin (MBL), a C-type lectin with a collagen-like domain (Kawasaki et al., 1983), is an acute-phase protein. MBL binds mannose and *N*-acetyl-D-glucosamine sugars expressed on a wide range of pathogens including Gram-positive and Gram-negative bacteria, yeast, parasites, and viruses (Takahashi et al., 2005). On binding to its ligands, MBL activates the complement via MBL-associated serine protease (MASP)-1 and MASP-2 in an antibody-independent manner. In addition, MBL opsonizes bacteria using the C1q receptor on macrophages without involving a complement (Nepomuceno et al., 1997), regulates inflammatory cytokines released by phagocytic cells, and inhibits virus infectivity (Wakamiya et al., 1992).

Most mammals have 2 *MBL* genes, *MBL1* and *MBL2* (Kawai et al., 1997; Agah et al., 2001; Ma et al., 2007), which encode the MBL-A and MBL-C proteins, respectively. Both *MBL1* and *MBL2* mutations have been shown to vary the susceptibility of animals to various infections (Lillie et al., 2005). Impaired disease resistance has been associated with 3 single-nucleotide polymorphisms (SNPs) within the coding region of *MBL1* in various breeds of pigs (Lillie et al., 2006). Studies of human MBL-C have revealed that 3 SNPs in the collagen-like domain and 3 additional mutations within the *MBL2* promoter region can affect the assembly or formation of MBL-C, thus contributing to a low level of plasma MBL and innate immune dysfunction (Madsen et al., 1994, 1998; Capparelli et al., 2008). Accumulating data have revealed that haplotype combinations of human *MBL2* SNPs generate increasing susceptibility to various bacterial, viral, and parasitic diseases as well as to systemic lupus erythematosus, rheumatoid arthritis, hepatitis, cystic fibrosis, and ischemia-reperfusion injury (Eisen and Minchinton, 2003; Holmskov et al., 2003; Takahashi et al.,

2005). A case-control study has demonstrated that polymorphism at the *MBL2* locus of water buffalo is associated with susceptibility and resistance to *Brucella abortus* infection (Capparelli et al., 2008).

Chinese Holsteins, named in 1992, are derived from grading crossbreeding and selection between native cows and purebred Holstein bulls introduced to China (Qiu, 2002). Given the possible association between the *MBL2* polymorphism and resistance to pathogen infection and susceptibility, which has been confirmed in water buffalo, we scanned the *MBL2* gene in Chinese Holsteins to determine whether the polymorphisms are associated with SCS and milk performance traits.

MATERIAL AND METHODS

Animals

The 825 Chinese Holstein cows (age ranging from 4 to 7 years, including first to fourth parity) in the study group were from 7 farms in China. Milk samples were taken from each cow once each month for an entire lactation period. Data of milk performance traits (305-day matured equivalency, fat percentage, protein percentage, and SCS) were collected from the laboratory of a dairy herd improvement center, OX Biotechnology, Shandong, China, using a milk composition analyzer (Foss Milk Scan FT 6000, Denmark) for statistical analysis. The mean and standard error of 305-day matured equivalency, fat percentage (%), protein percentage (%), and SCS were 6319.61 ± 342.35 kg, 3.91 ± 0.11%, 3.22 ± 0.06%, and 4.08 ± 0.78, respectively, in the tested animals.

Detection of *MBL2* polymorphisms

Blood samples of the cows were collected from the jugular vein and mixed with acid-citrate-dextrose anticoagulant (0.48% citric acid, 1.32% citrate sodium, and 1.47% dextrose) at a ratio of 6:1 (blood:anticoagulant), and stored at -20°C. Genomic DNA was extracted from the blood using a phenol-chloroform protocol proposed by Huang et al. (2010). DNA content was quantified spectrophotometrically and diluted to 50 ng/μL.

Primer pair P1 (5'-TAATCTCCCTTGACCTTTCTTACACC-3') and P2 (5'-AGAACA GCCCAAACTGT-3') were designed using the Primer V 5.0 software to amplify a 247-bp fragment at exon 1 of the bovine *MBL*2 (GenBank accession No. NC_007327.3). Polymerase chain reaction (PCR) was carried out in a final volume of 25 μL. The reaction contained 50 ng template DNA, 0.5 μM of each primer, 0.5 mM deoxyribonucleotide triphosphates, 1.8 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (TaKaRa, Dalian, China). After an initial denaturation at 94°C for 5 min, PCR amplification was performed in 35 cycles using the following parameters: denaturation at 94°C for 30 s, annealing at 57.8°C for 30 s, and elongation at 72°C for 30 s. The reaction was continued for a final extension at 72°C for 8 min. PCR products were electrophoresed on 1% agarose gels.

The single-strand conformational polymorphism (SSCP) method was used to scan mutations within the amplified regions. Five microliters of the PCR products of each individual was mixed with 5 μL denaturing solution (98% formamide, 25 mM ethylenediaminetetraacetic acid, 0.025% xylene-cyanol FF, and 0.025% bromophenol blue), heated

at 98°C for 10 min, and rapidly chilled on ice for 5 min. Denatured DNA was subjected to 10% polyacrylamide gel electrophoresis in 1X Tris-borate-ethylenediaminetetraacetic acid buffer at a constant voltage (120 V) and a constant temperature (4°C) for 12 h. The gel was stained with 0.1% silver nitrate.

Nucleotide sequencing

Generally, the PCR products amplified from genomic DNA and the PCR products of various SSCP electrophoresis patterns were directly sequenced in both directions in an ABI PRISM 3730 DNA analyzer (Applied Biosystems) and the sequences were analyzed with DNASTAR 5.0.

Statistical analysis

The distribution frequency of somatic cell count (SCC) is usually skewed. A parameter based on SCC that is often used is SCS. Cow SCS is calculated as SCS = $\log_2$ (SCC / 100) + 3, where SCC is calculated as cells per microliter (Rupp and Boichard, 1999). Genotypic and allelic frequencies, value of chi-square, polymorphism information content (PIC), expected heterozygosity (H_E), and effective number of alleles (N_E) were analyzed using the TFPGA software. The linkage disequilibrium and haplotype analysis were performed using the SHEsis software (Shi and He, 2005). The association between SNP marker genotypes of *MBL2* and SCS were analyzed using the least-squares method as applied in the general linear model procedure of SAS (SAS Institute Inc., Cary, NC, USA) according to the following:

$$Y_{ijklm1} = \mu + G_i + S_j + H_k + F_l + e_{ijklm}$$

where Y_{ijklm1} is the observed value; μ is the overall mean; G_i is the effect of genotype or combined genotype; S_j is the effect of season; H_k is the effect of parity; F_l is the effect of farm, and e_{ijklm} is the random error. Values of $P < 0.05$ were considered to be significant.

RESULTS

SNPs of *MBL2* in Chinese Holsteins

SSCP gels generated 9 distinct banding patterns for exon 1 of *MBL2* from the 825 Chinese Holsteins studied (Figure 1). Nucleotide sequencing corresponding to the various banding patterns revealed the nucleotide characterizations of the amplified region. The variations of 4 nucleotides were detected in *MBL2* exon 1 at positions of g.1164 G>A, g.1197 C>A, g.1198 G>A, and g.1207 T>C in comparison to the GenBank sequence (see Figure 2). The g.1164 G>A (codon 31) SNP was predicted to substitute arginine with glutamine at the N-terminus of the cysteine-rich domain, and g.1197 C>A and g.1198 G>A (codon 42) changed proline to glutamine in the first Gly-X-Y repeat of the collagen-like domain, whereas g.1207 T>C (codon 45) was identified as a synonymous mutations [AAT (Asn) > AAC (Asn)] at the position of the 45th amino acid in the collagen-like domain (Figure 3).

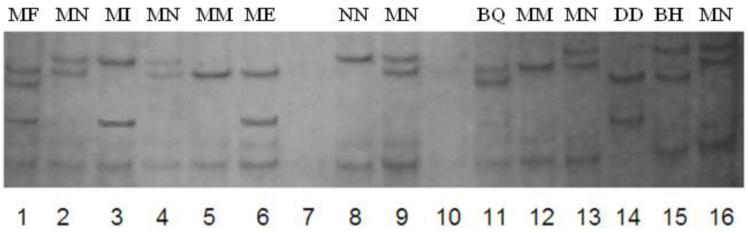

Figure 1. Silver-stained single-strand conformational polymorphism (SSCP) polyacryamide gel of exon 1 of the *MBL2* gene in Chinese Holstein. Nine banding patterns were visualized corresponded with g.1164 G>A, g.1197 C>A, g.1198 G>A, and 1207 T>C for all 825 Chinese Holstein analyzed by SSCP. Nine haplotype pairs of g.1164 G>A, g.1197 C>A, g.1198 G>A and 1207 T>C SNPs were found in the cattle *MBL2* gene, named MM = GG/CC/GG/TT; MN = AG/CC/GG/TT; BQ = GG/CC/AG/TC; NN = AA/CC/GG/TT; BH = AG/CC/AG/TC; DD = GG/CC/AA/CC; ME = GG/AC/GG/TT; MI = AG/AC/GG/TT; MF = GG/AC/AG/TT.

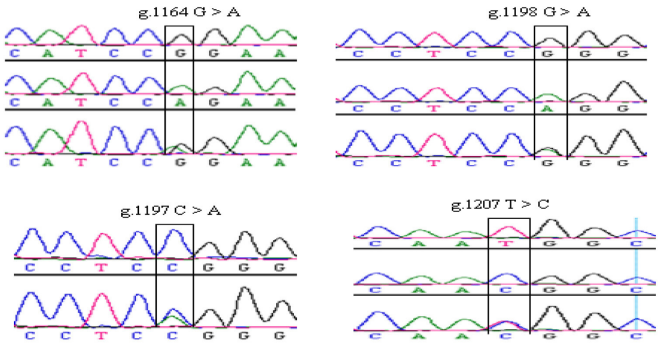

Figure 2. Sequencing analysis on exon 1 of the *MBL2* gene in Chinese Holstein.

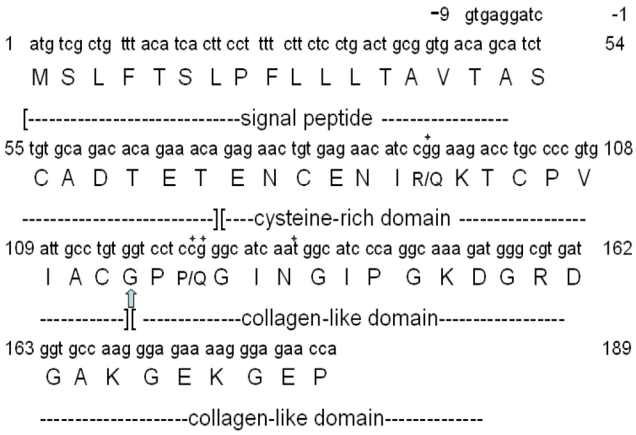

Figure 3. Full-length sequence of exon 1 of the bovine MBL2 analyzed by single-strand conformational polymorphism, including the entire signal peptide and cysteine-rich domain and part collagen-like domain as well as 5' (-1 to -9) (GenBank accession No. NC_007327). Asterisks indicate the locations of the single nucleotide polymorphisms (SNPs) identified at positions g.1164 G > A, g.1197 C>A, g.1198 G>A and g.1207 T>C. The SNP at position 1164 encodes an amino acid substitution of Gln for Arg. The SNPs g.1197 C>A and g.1198 G>A made Pro to Gln. Numbers indicate amino (left side) and nucleotide (right side) position from the start codon. Arrowhead indicates restriction endonuclease cleavage sites.

PCR-SSCP and allele frequencies

A genetic index was evaluated in Chinese Holsteins. The value of the chi-square test, H_E, N_E, PIC, and allele and genotype frequencies are presented in Table 1. The genetic index showed that the g.1164 G>A, g.1197 C>A, g.1198 G>A, and g.1207 T>C polymorphisms had the lowest levels of genetic polymorphisms in the tested individuals (Table 1) according to the criterion of PIC. Alleles G, C, G, and T in the mutational sites g.1164 G>A, g.1197 C>A, g.1198 G>A, and g.1207 T>C were predominant in the Chinese Holsteins.

Table 1. Expected heterozygosity (H_E), χ^2 test, effective number of alleles (N_E), and polymorphism information content (PIC) of the *MBL2* gene in Chinese Holstein cows.

SNP	Genotype	Samples	Genotypic frequency	Allele	Allelic frequency	PIC	H_E	N_E	χ^2	P
g.1164 G>A	GG	581	0.7042	G	0.8024	0.2668	0.3171	1.4643	119.57	0.0000
	GA	162	0.1964							
	AA	82	0.0994	A	0.1976					
g.1197 C>A	CC	785	0.9515	C	0.9758	0.0462	0.0473	1.0497	0.5092	0.4755
	CA	40	0.0485	A	0.0242					
g.1198 G>A	GG	538	0.6521	G	0.7709	0.2908	0.3532	1.5461	88.42	0.0000
	GA	196	0.2376							
	AA	91	0.1103	A	0.2291					
g.1207 T>C	TT	564	0.6836	T	0.7970	0.2712	0.3236	1.4785	74.04	0.0000
	TC	187	0.2267							
	CC	74	0.0897	C	0.2030					

Relationships between SNPs and haplotype pairs of *MBL2* and bovine milk production traits

The effects of the 4 *MBL2* loci on milk production traits and SCS are shown in Table 2. Correlation analysis showed that 2 of the SNPs (g.1164 G>A and g.1197 C>A) were significantly correlated with SCS ($P < 0.05$). As shown in Table 2, milk from cows with genotype GG had higher fat content and protein content and lower SCS than that of genotype GA in site g. 1164 G>A. Compared with that of cows with genotype CC, milk of genotype CA had significantly higher SCS in mutational site g.1197 C>A but no significant differences in other traits. No significant correlations were observed between any of the marker genotypes in mutational sites (g.1198 G>A and g.1207 T>C) and milk production traits and SCS.

Table 2. Least squares mean and standard errors for milk production traits of different *MBL2* genotypes in 825 Chinese Holstein cows.

Loci	Genotype	Somatic cell score	Fat content (%)	Protein content (%)	Fat yield (kg)	Protein yield (kg)	305-day milk yield (kg)
g.1164 G>A	GG	3.01 ± 0.43^b	4.08 ± 0.21^a	3.23 ± 0.05^a	191.64 ± 80.03	142.47 ± 16.90	6497.49 ± 213.43
	GA	3.83 ± 0.40^a	3.70 ± 0.13^b	3.15 ± 0.05^b	160.00 ± 87.65	156.95 ± 18.51	6549.70 ± 231.18
	AA	4.56 ± 0.99^a	3.91 ± 0.12	3.19 ± 0.11	140.56 ± 199.53	121.09 ± 42.14	6057.96 ± 470.14
g.1197 C>A	CC	3.63 ± 0.38^B	3.80 ± 0.11	3.22 ± 0.05	184.11 ± 78.54	146.46 ± 16.63	6511.51 ± 209.01
	CA	5.38 ± 0.52^A	3.72 ± 0.13	3.19 ± 0.07	143.27 ± 106.79	146.69 ± 22.61	6385.34 ± 331.76
g.1198 G>A	GG	3.63 ± 0.40	3.87 ± 0.11	3.25 ± 0.06	182.11 ± 80.79	145.12 ± 16.46	6567.74 ± 211.38
	GA	3.82 ± 0.47	3.90 ± 0.11	3.22 ± 0.05	179.29 ± 94.94	146.14 ± 19.34	6333.58 ± 238.08
	AA	5.00 ± 1.28	3.88 ± 0.12	3.19 ± 0.05	198.13 ± 56.92	147.08 ± 52.34	6078.58 ± 429.83
g.1207 T>C	TT	3.642 ± 0.40	3.75 ± 0.13	3.21 ± 0.09	181.41 ± 80.86	144.83 ± 16.47	6569.88 ± 211.29
	TC	3.66 ± 0.48	3.84 ± 0.11	3.19 ± 0.06	184.05 ± 96.46	148.74 ± 19.65	6320.81 ± 239.37
	CC	5.00 ± 1.28	3.88 ± 0.13	3.20 ± 0.07	198.98 ± 87.36	147.07 ± 52.42	5900.29 ± 438.94

Mean values with the different superscript lower case letters in the same mutational site and column denote significant difference, $P < 0.05$; with the different superscript upper case letters denote significant difference, $P < 0.01$.

Nine haplotypes - M = G/C/G/T, B = G/C/G/C, Q = G/C/A/T, D = G/C/A/C, E = G/A/G/T, F = G/A/A/T, N = A/C/G/T, H = A/C/A/T, and I = A/A/G/T - corresponding to the loci of the 4 novel SNPs were found in the 825 Chinese Holsteins using PCR-SSCP analysis and sequencing. The estimated haplotype frequencies were 0.5327, 0.1255, 0.0921, 0.0400, 0.0206, 0.0036, 0.1206, 0.0667, and 0.0072, respectively. M showed the highest haplotype frequency, and F had the lowest haplotype frequency. Only 9 haplotype pairs - MM = GG/CC/GG/TT, MN = AG/CC/GG/TT, BQ = GG/CC/AG/TC, NN = AA/CC/GG/TT, BH = AG/CC/AG/TC, DD = GG/CC/AA/CC, ME = GG/AC/GG/TT, MI = AG/AC/GG/TT, and MF = GG/AC/AG/TT - at the loci of the 4 SNPs of *MBL2* were found (see Figure 1), although 64 haplotype combinations may be possible in the tested population. With respect to SCS, significantly fewer animals had haplotype pairs MM and BQ than had pairs BH, MI, and MF; haplotype pairs MI and MF were markedly more frequent than were haplotype pairs MN. With respect to protein yield, cows with haplotype pairs MN were significantly more frequent than those with MM and ME; cows with haplotype pairs BQ were significantly higher than those with haplotype pairs ME. With respect to 305-day milk yield, cows with haplotype pairs MN had noticeably higher yield than that of cows with haplotype pairs MM, BQ, and DD, respectively. Compared to the milk from cows with haplotype pairs MM and MN, the milk from cows with the other haplotypes had lower frequent protein contents. However, no significant difference in fat content and fat yield was observed among the various haplotype combinations (Table 3).

Table 3. Least squares mean and standard error for milk production traits of different *MBL2* haplotype pairs in 825 Chinese Holstein cows.

Trait genotype (No.)	Somatic cell score	Fat content (%)	Protein content (%)	Fat yield (kg)	Protein yield (kg)	305-day milk yield (kg)
MM (344)	3.57 ± 0.41^{abc}	4.606 ± 0.12	3.32 ± 0.05^{a}	196.77 ± 84.49	136.16 ± 17.039	6333.12 ± 223.92^{A}
MN (139)	3.76 ± 0.44^{de}	3.7 ± 0.11	3.17 ± 0.05^{b}	153.21 ± 91.22	159.94 ± 18.40^{a}	6828.42 ± 226.84^{ABc}
BQ (152)	3.58 ± 0.48^{fgh}	3.97 ± 0.11	3.20 ± 0.07	190.66 ± 98.41	144.25 ± 19.85	6306.47 ± 242.37^{B}
NN (30)	5.10 ± 1.25	3.72 ± 0.20	3.22 ± 0.05	159.23 ± 257.44	140.25 ± 51.92	6730.24 ± 589.11
BH (55)	5.51 ± 0.99^{af}	4.11 ± 0.19	3.22 ± 0.06	213.97 ± 205.13	167.42 ± 41.37	6071.19 ± 481.22
DD (33)	4.97 ± 1.26	3.87 ± 0.13	3.23 ± 0.10	105.17 ± 259.47	140.08 ± 52.32	5890.41 ± 437.57^{c}
ME (34)	4.57 ± 0.78	3.84 ± 0.13	3.20 ± 0.08	112.38 ± 160.77	83.50 ± 32.42^{b}	6244.71 ± 498.43
MI (12)	6.50 ± 1.26^{bdg}	3.79 ± 0.22	3.19 ± 0.12	140.56 ± 258.96	120.26 ± 52.22	5682.73 ± 868.85
MF (6)	6.24 ± 1.10^{ceh}	3.84 ± 0.21	3.20 ± 0.13	151.86 ± 225.46	118.94 ± 45.4652	6916.52 ± 756.94

Mean values with the same superscript lower case letters in the same column denote significant difference, $P < 0.05$; with the same superscript upper case letters in the same column denote significant difference, $P < 0.01$; means marked with different superscript or without any letters denote no significant difference. MM = GG/CC/GG/TT; MN = AG/CC/GG/TT; BQ = GG/CC/AG/TC; NN = AA/CC/GG/TT; BH = AG/CC/AG/TC; DD = GG/CC/AA/CC; ME = GG/AC/GG/TT; MI = AG/AC/GG/TT; MF = GG/AC/AG/TT.

DISCUSSION

Bovine *MBL2* is located on chromosome 26 (Gjerstorff et al., 2004) and contains 3 introns and 4 exons encoding 249 amino acids (see Figure 3). In the present study, 4 novel SNPs were found in the Chinese Holstein *MBL2* exon 1, located at the N-terminus of the cysteine-rich domain and Gly-X-Y repeat of the collagen-like domain of bovine *MBL-C* (see Figure 3). This report is the first to date on the Chinese Holstein *MBL2*.

The SNPs g.1164 G>A, g.1197 C>A, and g.1198 G>A may be the loci of the causative polymorphisms, as they are non-synonymous nucleotide polymorphisms at coding regions

affecting protein function. This postulation is supported by the following: first, the basic structure of *MBL-C* in humans and other mammals is a trimer of 3 identical monomers. A small cysteine-rich domain at the N-terminus forms a disulfide bridge between the 3 monomers, stabilizing the trimer. These cysteinyl residues are also critical for the formation of higher-order oligomers, with a minimum of 2 cysteines required for multimerization, although unknown factors also play a role in multimer stabilization (Brown-Augsburger et al., 1996; Ohashi and Erickson, 2004; Jensen et al., 2005). Second, *MBL-C* deficiency in humans and other mammals is caused predominantly by amino acid substitutions - that is, SNPs in exon 1 of *MBL2*. The substitution of arginine by glutamine, which occurs in codon 31 at the N-terminus of the cysteine-rich domain of *MBL2* in both the human and the rat *MBL-C*, also changes the disulfide bond arrangements (Larsen et al., 2004; Wallis, 2004) and is implicated in decreased ligand-binding activity and complement activation. Finally, adjacent to the N-terminal domain is a large collagen-like domain that forms the stem of collagenous lectins. It is related to the activation of the lectin complement pathway and opsonic immunity. The collagen-like domain of *MBL* is comprised of numerous Gly-X-Y repeats, and 3 monomers form a coiled-coil that provides stability to the tertiary structure (Lillie et al., 2005). Within this domain, several structural features are thought to contribute to the many distinct functions attributed to the collagen-like domain of mannan-binding lectin. The N-terminus of the collagen-like domain, including a conserved *GEKGEP* motif involved in interactions with the C1q receptor, is implicated in opsonophagocytosis (Arora et al., 2001). Meanwhile, within the C-terminus of the collagen-like domain are several conserved amino acid residues that form a putative MASP-binding motif (Wallis et al., 2004) and the assembly of the *MBL* trimer (Larsen et al., 2004). Various SNPs that replace amino acids in the collagen domain might alter the function of *MBL2*. In humans, 3 SNPs have been identified in *MBL2* exon 1 at codons 52 (Arg→Cys), 54 (Gly→Asp), and 57 (Gly→Glu) (Madsen et al., 1994), demonstrating an association with many innate immunological factors (Holmskov et al., 2003). The mutations in exon 1 of the human *MBL2* gene are frequently described as being associated with *MBL-C* plasma concentration, reduced ligand-binding capacity, and failure in complement activation (Larsen et al., 2004).

MBL mutations have also been predicted to contribute to varied susceptibility to infection (Lillie et al., 2005; Takahashi et al., 2005). One G→T SNP at position 271 (codon 91) of the pig *MBL-A* complementary DNA encodes a substitution of glycine with cysteine in the 16th Gly-X-Y repeat in the collagen-like domain, following the putative *MASP*-binding domain. Comparison of the frequency of the *MBL-A* 271 G→T mutation in healthy and diseased populations has revealed no significant difference in mutant allele (T) frequency or mutant-positive values. However, a marked tendency for the frequency of mutation in diseased populations to be higher than that in healthy populations does exist. It can be postulated that mutations by a glycine substitution in the 16th Gly-X-Y repeat of the collagen-like domain in trimeric *MBLs* could interfere with *MBL* structure in heterozygotes, thus decreasing MBL serum levels and increasing disease susceptibility (Lillie et al., 2006).

Results of the chi-square test of our data indicated significant differences in genotypic and allelic distributions within g.1164 G>A, g.1197 C>A, g.1198 G>A, and g.1207 T>C in the studied Holsteins. The number of g.1164 G>A and g.1197 C>A homozygotes was low or even undetectable, suggesting that the polymorphism may be associated with disease resistance.

Variations of both *MBL1* and *MBL2* have been associated with resistance to infections

such as *S. aureus* in mice (Shi et al., 2004), *K88+ E. coli*, *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, *Mycoplasma* spp, and circovirus 2 in pigs (Lillie et al., 2007), *B. abortus* in water buffalo (Capparelli et al., 2008), *Neorickettsia risticii* and *Clostridium* spp in horses (Podolsky et al., 2006), and meningococcal and *E. coli* infections in humans (Neth et al., 2000; Eisen and Minchinton, 2003; Smithson et al., 2007). Because bacteria are the most common causes of mastitis in cows, analysis of bovine *MBL2* SNPs may contribute to the discovery of their association with mastitis resistance. Statistical results showed that cows with the g.1164 G>A-GG and g.1197 C>A-CC genotypes had the significant lower SCS, indicating that these genotypes might be associated with mastitis resistance in the population. Therefore, cattle with genotypes GG and CC can be selected for breeding.

No significant correlations were observed between any of the marker genotypes in mutational sites (g.1198 G>A and g.1207 T>C) and milk production traits or SCS. Interestingly, a significant association existed between the combined genotypes of 4 SNPs (g.1164 G>A, g.1197 C>A, g.1198 G>A, and g.1207 T>C) and milk production traits (protein rate, 305-day milk yield, and SCS but not fat rate and fat yield), suggesting that one SNP may be influenced by others. Thus, the analysis of haplotypes in marker-assisted selection is more valuable than that of SNPs. Use of haplotypes with a strong causal relationship to the trait under study is a characteristic common to a long series of successful and repeatedly confirmed case-control studies (Risch, 2000). To our knowledge, our report is the first on haplotype and haplotype pairs in bovine *MBL2*. Our results suggested that Chinese Holsteins with MM and BQ haplotype pairs at bovine *MBL2* exon 1 had lower SCS and a lower risk for developing mastitis owing to contagious and environmental pathogens. Although the bonding of *S. aureus* (contagious pathogen) and *E. coli* (environmental pathogen) to *MBL-C* was not measured for technical reasons, the relationship between *MBL2* genotypes and *E. coli* and *B. abortus* resistance has been clearly established (Neth et al., 2000; Smithson et al., 2007; Capparelli et al., 2008). Therefore, *MBL2* haplotyping could be of interest to breeders as a molecular marker predisposing to mastitis. This conclusion is consistent with that of Fallin et al. (2001). Positive correlations may exist between the 305-day milk yield with haplotype pairs MN, mastitis resistance with haplotype pairs MM, MN, and BQ, protein yield with haplotype pairs MN, and protein rate with haplotype pairs MM. Haplotype pairs are convincing molecular markers for future selections for high milk yields, mastitis resistance, and high protein rates in cattle.

ACKNOWLEDGMENTS

Research supported by the National Cow Anti-TB Transgenic Project (#2008ZX08007-004), the "863" Project of the Chinese Ministry of Science and Technology (#2006AA10Z169), the National Cow Industrial Technology System Program (#nycytx-0107, #nyhyzx07-036-09), and the Key Scientific and Technological Project from Shandong Province (#2009GG20002033).

REFERENCES

Agah A, Montalto MC, Young K and Stahl GL (2001). Isolation, cloning and functional characterization of porcine mannose-binding lectin. *Immunology* 102: 338-343.

Arora M, Munoz E and Tenner AJ (2001). Identification of a site on mannan-binding lectin critical for enhancement of phagocytosis. *J. Biol. Chem.* 276: 43087-43094.

Brown-Augsburger P, Hartshorn K, Chang D, Rust K, et al. (1996). Site-directed mutagenesis of Cys-15 and Cys-20 of

pulmonary surfactant protein D. Expression of a trimeric protein with altered anti-viral properties. *J. Biol. Chem.* 271: 13724-13730.

Capparelli R, Parlato M, Amoroso MG, Roperto S, et al. (2008). Mannose-binding lectin haplotypes influence *Brucella abortus* infection in the water buffalo (*Bubalus bubalis*). *Immunogenetics* 60: 157-165.

Chaneton L, Tirante L, Maito J, Chaves J, et al. (2008). Relationship between milk lactoferrin and etiological agent in the mastitic bovine mammary gland. *J. Dairy Sci.* 91: 1865-1873.

Eisen DP and Minchinton RM (2003). Impact of mannose-binding lectin on susceptibility to infectious diseases. *Clin. Infect. Dis.* 37: 1496-1505.

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.

Gjerstorff M, Hansen S, Jensen B, Dueholm B, et al. (2004). The genes encoding bovine SP-A, SP-D, MBL-A, conglutinin, CL-43 and CL-46 form a distinct collectin locus on *Bos taurus* chromosome 28 (BTA28) at position q.1.8-1.9. *Anim. Genet.* 35: 333-337.

Holmskov U, Thiel S and Jensenius JC (2003). Collections and ficolins: humoral lectins of the innate immune defense. *Annu. Rev. Immunol.* 21: 547-578.

Huang J, Wang H, Wang C, Li J, et al. (2010). Single nucleotide polymorphisms, haplotypes and combined genotypes of lactoferrin gene and their associations with mastitis in Chinese Holstein cattle. *Mol. Biol. Rep.* 37: 477-483.

Jensen PH, Weilguny D, Matthiesen F, McGuire KA, et al. (2005). Characterization of the oligomer structure of recombinant human mannan-binding lectin. *J. Biol. Chem.* 280: 11043-11051.

Kawai T, Suzuki Y, Eda S, Ohtani K, et al. (1997). Cloning and characterization of a cDNA encoding bovine mannan-binding protein. *Gene* 186: 161-165.

Kawasaki N, Kawasaki T and Yamashina I (1983). Isolation and characterization of a mannan-binding protein from human serum. *J. Biochem.* 94: 937-947.

Larsen F, Madsen HO, Sim RB, Koch C, et al. (2004). Disease-associated mutations in human mannose-binding lectin compromise oligomerization and activity of the final protein. *J. Biol. Chem.* 279: 21302-21311.

Lillie BN, Brooks AS, Keirstead ND and Hayes MA (2005). Comparative genetics and innate immune functions of collagenous lectins in animals. *Vet. Immunol. Immunopathol.* 108: 97-110.

Lillie BN, Keirstead ND, Squires EJ and Hayes MA (2006). Single-nucleotide polymorphisms in porcine mannan-binding lectin A. *Immunogenetics* 58: 983-993.

Lillie BN, Keirstead ND, Squires EJ and Hayes MA (2007). Gene polymorphisms associated with reduced hepatic expression of porcine mannan-binding lectin C. *Dev. Comp. Immunol.* 31: 830-846.

Ma BY, Nakamura N, Dlabac V, Naito H, et al. (2007). Isolation, cloning, and characterization of a novel phosphomannan-binding lectin from porcine serum. *J. Biol. Chem.* 282: 12963-12975.

Madsen HO, Garred P, Kurtzhals JA, Lamm LU, et al. (1994). A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* 40: 37-44.

Madsen HO, Satz ML, Hogh B, Svejgaard A, et al. (1998). Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. *J. Immunol.* 161: 3169-3175.

Nepomuceno RR, Henschen-Edman AH, Burgess WH and Tenner AJ (1997). cDNA cloning and primary structure analysis of C1qR(P), the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis *in vitro*. *Immunity* 6: 119-129.

Neth O, Jack DL, Dodds AW, Holzel H, et al. (2000). Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect. Immun.* 68: 688-693.

Ohashi T and Erickson HP (2004). The disulfide bonding pattern in ficolin multimers. *J. Biol. Chem.* 279: 6534-6539.

Podolsky MJ, Lasker A, Flaminio MJ, Gowda LD, et al. (2006). Characterization of an equine mannose-binding lectin and its roles in disease. *Biochem. Biophys. Res. Commun.* 343: 928-936.

Qiu H (2002). Modern Dairy Science. Agriculture Press, China, 1-12.

Risch NJ (2000). Searching for genetic determinants in the new millennium. *Nature* 405: 847-856.

Rupp R and Boichard D (1999). Genetic parameters for clinical mastitis, somatic cell score, production, udder type traits, and milking ease in first lactation Holsteins. *J. Dairy Sci.* 82: 2198-2204.

Seegers H, Fourichon C and Beaudeau F (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res.* 34: 475-491.

Shi L, Takahashi K, Dundee J, Shahroor-Karni S, et al. (2004). Mannose-binding lectin-deficient mice are susceptible to infection with *Staphylococcus aureus*. *J. Exp. Med.* 199: 1379-1390.

Shi YY and He L (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res.* 15: 97-98.

Smithson A, Munoz A, Suarez B, Soto SM, et al. (2007). Association between mannose-binding lectin deficiency and

septic shock following acute pyelonephritis due to *Escherichia coli*. *Clin. Vaccine Immunol.* 14: 256-261.

Takahashi R, Tsutsumi A, Ohtani K, Muraki Y, et al. (2005). Association of mannose binding lectin (*MBL*) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus. *Ann. Rheum. Dis.* 64: 311-314.

Wakamiya N, Okuno Y, Sasao F, Ueda S, et al. (1992). Isolation and characterization of conglutinin as an influenza A virus inhibitor. *Biochem. Biophys. Res. Commun.* 187: 1270-1278.

Wallis R, Shaw JM, Uitdehaag J, Chen CB, et al. (2004). Localization of the serine protease-binding sites in the collagen-like domain of mannose-binding protein: indirect effects of naturally occurring mutations on protease binding and activation. *J. Biol. Chem.* 279: 14065-14073.