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Brief report

A novel SNP of Lysozyme Gene and Its Association with Mastitis Trait in Chinese Holstein

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Background

Mastitis is the most frequent and important disease in the dairy industry worldwide, which leads to great economic losses in the dairy industry (Nash *et al.* 2003). Direct selection for resistance to clinical mastitis may be very difficult and indirect selection has been practiced widely. The recommended measure is to record herd milk somatic cell scores (SCS) based on the positive correlation between clinical mastitis and milk SCS (Rupp *et al.* 1999).

Lysozyme is a ubiquitous bacteriolytic enzyme present in external secretions and in polymorphonuclear leukocytes and macrophages. The lysozyme level is an index of macrophage functional status (Di Luzio 1979). Seyfert *et al.* (1996) suggested the *Lyz* gene as a candidate gene for improvement of mastitis resistance. The activity of lysozyme may be the result of single nucleotide change. The *Lyz* gene is located on autosome 5, containing 4 exons and 3 introns. The purposes of this study were to reveal single nucleotide polymorphisms (SNPs) in the encoding region of *Lyz* gene and to evaluate the possible association of SNPs with SCS in Chinese Holstein populations.

Abbreviations: PCR: polymerase chain reaction, PCR-SSCP: polymerase chain reaction single strand conformation polymorphism, SCC: somatic cell count, SCS: somatic cell score, SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SNP: single nucleotide polymorphism

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Procedures

Primer sequence

Following primer sequences were used:
P1 (forward) - 5'-CAGTCTGGACATTTGACTTCTCA-3',
P1 (reverse) - 5'-TTGCTCATTATT CATCCTCTGTT-3'.
P2 (forward) - 5'-GAACTGATTGGGCTATGAAGTGT-3',
P2 (reverse) - 5'-AGTATAACC AGATCGGGGCTGAG-3'.
P3 (forward) - 5'-GAAAATAAAGCCTGCTGAACCTA-3',
P3 (reverse) - 5'-TCAAACT CTTATACCGTACACCT-3'.
P4 (forward - 5'-GATGAACTATTTTGTTCTCCCAA-3',
P4 (reverse) - 5'-CTTTCA TTCAACTATCACTTCCT-3'

PCR amplification condition

Deoxyribonucleic acid was isolated from blood samples of 610 Chinese Holstein, the offspring of 30 bulls, using a phenol-chloroform extraction protocol followed by an ethanol precipitation step. Polymerase chain reaction (PCR) was carried out in a typical 20 µl system containing 100 ng template, 1 µl primer (10 pmol/µl), 0.4 µl dNTPs (10 mmol/µl), 1.0 µl MgCl2 (25 mmol), 1.5 U Taq DNA polymerase and 2 µl 10× buffer. Polymerase chain reaction amplification conditions were used as follows: pre-denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. The PCR products were detected on a 1.5 % agarose gel.

Detection of Lyz gene polymorphisms

The polymorphism of *Lyz* gene was detected by the method of PCR-Single Strand Conformation Polymorphism (PCR-SSCP). A total of 2.0 µl PCR product was mixed with 8 µl of the denaturation solution (50 mmol/l NaOH, 1 mmol/l EDTA) and 1 µl of the loading buffer, containing 0.25 % bromophenol blue and 0.25 % xylene cyannol, denatured for 10 min at 98 °C and rapidly chilled in –20 °C. The samples were then electrophoresed in 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A thermostatically controlled refrigerated circulator was used to maintain a constant temperature (4 °C) of the gels. The gels were run under the following conditions: 250 V, 40 mA, 10 min (pre-electrophoresis) and 150 V, 24 mA, for 8 h. The gels were then stained with Silver Stain (Kucharczyk Techniki Elektroforetyczne). The patterns of DNA bands were observed and photographed with the GDS7500 System (UVP Inc., Upland, CA, USA). After the polymorphism was detected, 4 samples of each type of band were sequenced and analysed.

Detection of Lysozyme concentration in milk

Twenty-four milk samples from the offspring were collected for the ELISA test (8 offspring from each genotype).

Statistical analysis

The frequencies of alleles and genotypes were analysed with POPGENE software (ver.1.31). The χ^2-test was to test linkage disequilibrium. All the production data were analysed with the GLM procedure of SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). The relation of the *Lyz* gene polymorphism with 305-d milk yield and somatic SCS were analysed with a general linear model:

$$y = \mu + b + ys + p + mm + cs + g + e \quad (1)$$

Where y is the individual phenotypic value, μ is the overall mean, b is the fixed effect for sire (30), ys is the fixed effect of year and season, p is the fixed effect of parity (1-4), mm is the fixed effect of milking month (day in milk, 1-12), cs is the fixed effect of calving season (3-5: spring, 6-8: summer, 9-11: autumn, 12-2: winter), g is the fixed effect of genotype and e is the random residual effect. The somatic cell count (SCC) was converted into the SCS (Wiggans *et al*. 1987) (SCS=log2 (SCC/100 000) +3) and rectified to eliminate the effect of lactation days and period of sampling on SCS (Houng *et al*. 2000).

Statistical significance of the parameter values among the different genotype was tested by ANOVA procedure. The treatment means were separated by Duncan's multiple range test and accepted if $P<0.05$.

Results

SNP identification and distributions of alleles and genotype

Results for PCR-SSCP showed that three types of SSCP bands were detected in primer 1. Direct DNA sequencing of the PCR productions of *Lys* gene revealed a single base nucleotide transversion from T to G at c. 115T>G site in the exon 1, resulting in replacement of Arg by Leu. The TT, TG and GG genotypes were designated, respectively. The genotypic and allelic frequencies of the mutations are shown in Table 1. The predominant allele was T (0.524) with TG presented at a high frequency (0.508) and GG presented at a low frequency (0.222). The χ^2 value was 288.11, indicating deviation from Hardy-Weinberg equilibrium in Chinese Holstein.

Table 1
The frequencies of genotype and allele and least squares mean and standard errors for SCS, 305 d milk yield and lysozyme concentration of different genotypic groups in Chinese Hostein

Locus	Geno-type	Genotype frequency	Allele	Allele frequency	χ^2 (P)	Least squares mean ± standard error		
						SCS	305 d milk yield, kg	Lysozyme concentration, μg/mL
115 T>G	TT	0.270	T	0.524	288.1 (0.000)	4.204 ± 0.075^{B}	$6\,067 \pm 93.37^{B}$	0.33 ± 0.041^{a}
	TG	0.508	G	0.476		4.160 ± 0.078^{b}	$6\,198 \pm 105.5^{b}$	0.38 ± 0.035
	GG	0.222				4.015 ± 0.066^{Aa}	$6\,418 \pm 110.27^{Aa}$	0.48 ± 0.02^{b}

Small letters means values in the same column with different letters significantly differ at $P<0.05$; capital letters means values in the same column with different letters highly significant differ at $P<0.01$

Associations between SNPs and SCS

The results of association analysis between different genotypes and SCS and 305-d milk yield traits are given in Table 1. The SNP Lys c.115T>G correlated significantly with SCS and 305-d milk yield. Least square mean of SCS for GG genotype was highly and significantly lower than that for TT ($P<0.01$) and significantly lower than the TG genotype ($P<0.05$). Least square mean of 305-d milk yield for genotype GG (6 418 ± 110.27) was greatly and significantly higher than that for TT (6 067 ± 93.37; $P<0.01$) and significantly higher than genotype TG (6 198 ± 105.5; $P<0.05$).

Lysozyme concentration of different genotypes in the cow's milk

The concentration of different genotypes in the cow's milk were measured with the ELISA method and the results showed that the GG genotype was significantly higher (0.48 ± 0.02) than the TT genotype (0.33 ± 0.041; $P<0.05$) and the TG genotype (0.38 ± 0.035) was higher than the TT genotype (0.33 ± 0.041).

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