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Short communication: Enhanced clinical mastitis resistance in Holsteins with a *FEZL* p.Gly105(12_13) polymorphism

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ABSTRACT

Mastitis is a common infectious disease of the mammary gland and a major problem in the dairy industry. We previously reported that forebrain embryonic zinc finger-like (*FEZL*) encoding a stretch of 12 glycines (p.Gly105[12]) instead of 13 glycines (p.Gly105[13]) is associated with a lower somatic cell score (SCS) in a family derived from Walkway Chief Mark. Here we report that the p.Gly105[12] allele is associated with a significantly decreased incidence of clinical mastitis in a large Holstein population. We genotyped the *FEZL* polymorphism in 918 randomly collected Holstein sires, and investigated the effect of the polymorphism on the estimated breeding value (EBV) for SCS and milk, fat, solids-not-fat, and protein yield, and on the number of cattle with clinical mastitis among daughters derived from these sires. The average EBV for SCS among sires carrying the heterozygous p.Gly105[12] was significantly lower than that among sires carrying the homozygous p.Gly105[13], whereas we found no unfavorable effects of this polymorphism on EBV for milk, fat, solids-not-fat, and protein yield. The proportion of cows with clinical mastitis derived from sires carrying heterozygous p.Gly105[12] was significantly lower than that of daughters derived from sires carrying the homozygous p.Gly105[13]. Thus, selection of sires carrying p.Gly105[12] could be beneficial in the dairy industry by reducing the incidence of mastitis.
Key words: cattle, mastitis, forebrain embryonic zinc finger-like, somatic cell score

Short Communication

Mastitis is an inflammation of the mammary gland caused by bacteria such as *Escherichia coli* that generates large losses in the dairy industry due to reductions in milk quality and quantity and increased health costs. Recently, linkage analysis of granddaughters derived from Walkway Chief Mark with high and low SCS during their first lactation period revealed that high SCS cows have a forebrain embryonic zinc finger-like (**FEZL**) protein with a longer glycine stretch; that is, 13 glycines (p.Gly105[13]) instead of 12 (p.Gly105[12]; Sugimoto et al., 2006). The FEZL protein is a transcription factor containing C2H2-type zinc-finger domains and a glycine stretch (Matsuo-Takasaki et al., 2000). Treatment of bovine mammary epithelial cells with LPS induces *FEZL* expression followed by enhanced production of tumor necrosis factor-α and IL-8 through semaphorin 5A expression (Sugimoto et al., 2006). Because p.Gly105[12] promotes higher semaphorin 5A expression than p.Gly105[13], the high SCS might be due to an impaired immune response of cows carrying p.Gly105[13].

The *FEZL* gene was mapped as influencing SCS in a large family derived from a specific sire, Walkway Chief Mark (Sugimoto et al., 2006); a strong genetic correlation exists between SCS and mastitis (Young et al., 1960; Emanuelson et al., 1988). The effects of the *FEZL* mutation on SCS and the incidence of mastitis, however, must be confirmed among randomly collected samples before genetic selection based on this gene can be implemented in the dairy industry. The aim of this study was to determine the effect of p.Gly105[12] on resistance to mastitis.

For genotyping of the *FEZL* p.Gly105(12_13) mutation, 918 DNA samples were prepared from semen according to standard protocols, and the DNA concentration was adjusted to 20 ng/μL. The PCR reaction was performed in a volume of 15 μL containing 20 ng of genomic DNA, 1.67 m*M* $MgCl_2$, 6.25 pmol of each primer [forward: 5′(FAM)-ACTCTGAGCTCTGGAAAAGCAG-3′; reverse: 5′-CACACGCCACAAGTTGGTTT-3′], 0.2 m*M* deoxynucleotides, and 0.375 U of *Taq* DNA polymerase (ABgene, Epsom, UK). The thermal cycling conditions were 1 cycle (94°C for 3 min), 35 cycles (94°C for 1 min, 60°C for 1 min, 72°C

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Table 1. The average ± SEM EBV for SCS, milk, fat, SNF, and protein yield of sires with heterozygous p.Gly105[12] compared with sires with homozygous p.Gly105[13] for the FEZL p.Gly105(12_13) mutation

Sire genotype	SCS	Milk, kg	Fat, kg	SNF, kg	Protein, kg
Heterozygous p.Gly105[12]	2.46 ± 0.04	43.3 ± 86.6	−0.44 ± 2.61	1.08 ± 6.81	−1.64 ± 2.28
Homozygous p.Gly105[13]	2.60 ± 0.01	83.5 ± 28.7	−1.59 ± 0.86	4.38 ± 2.30	−0.12 ± 0.78
P-value[1]	0.004	0.679	0.696	0.678	0.564

[1]Calculated by Student's *t*-test.

for 1 min), and 1 cycle (72°C for 10 min). Following PCR, alleles were resolved using an ABI 3700 sequencer (Applied Biosystems, Foster City, CA) and genotype data were captured using GeneMapper 4.0 (Applied Biosystems).

Genotyping of *FEZL* in 918 sires revealed that 97 sires had a heterozygous p.Gly105[12] genotype and 821 sires had a homozygous p.Gly105[13] genotype. The National Livestock Breeding Center (Fukushima, Japan) evaluated the EBV for SCS of 693 sires having more than 15 daughters among these 918 sires using a mixed model including a fixed regression:

$$y = HD + A + at + b \times \exp(-0.05t) + u + pe + e,$$

where y = SCS of first calving, HD = fixed effects of herd-test day, A = fixed effects of calving age groups, t = days in milk, a and b = coefficients of Wilmink's curves, u = random effects of additive genetics (EBV) and $u \sim N\left(0, \mathbf{A}\sigma_u^2\right)$, pe = random effects of permanent environment, e = random residuals, and $e \sim N\left(0, \mathbf{A}\sigma_e^2\right)$, where $\mathbf{A}$ is the numerator relationship matrix among animals. Heritability for SCS was 0.082. The average EBV for SCS of the heterozygous p.Gly105[12] sires (n = 59) was 2.47 ± 0.04, and the average EBV for SCS of the homozygous p.Gly105[13] sires (n = 634) was 2.60 ± 0.01. Student's *t*-test revealed a difference between these 2 groups (Table 1). These findings confirmed that the p.Gly105[12] allele decreases SCS, not only in the Walkway Chief Mark family, but also in other families in Japan.

To estimate the effect of FEZL on the EBV for milk, fat, SNF, and protein yields, we compared the average EBV of heterozygous p.Gly105[12] sires (n = 59) and homozygous p.Gly105[13] sires (n = 634). The National Livestock Breeding Center calculated the EBV of each sire for milk, fat, SNF, and protein yield based on a single-trait animal model and a BLUP procedure. The relative EBV for milk, fat, SNF, and protein yields of homozygous p.Gly105[13] sires were not different from those of heterozygous p.Gly105[12] sires (Table 1). These findings confirmed that the p.Gly105[12] allele does not have unfavorable effects on milk yield.

To determine the effect of FEZL on the incidence of mastitis, we compared the number of cows with mastitis among daughters derived from sires carrying heterozygous p.Gly105[12] or homozygous p.Gly105[13] for each year of age. The Veterinary Clinical Center, Tokachi NOSAI (Hokkaido, Japan), recorded clinical mastitis in the Tokachi area of Hokkaido from 2003 to 2007. Cows diagnosed with mastitis and treated at least once per lactation by veterinarians were considered affected. The Holstein Cattle Association of Japan, Hokkaido Branch, sorted the cows according to their sires and age. They collected 132,210 mastitis records in 491,725 lactation periods from 228,945 cows during these 5 yr in this area. A total of 41,431 records in 162,655 lactation periods were from daughters of the 918 genotyped sires.

In daughters younger than 3 yr old, 2,136 (18.9%) among 11,283 daughters derived from sires carrying heterozygous p.Gly105[12] were affected, whereas 12,691 (20.1%) among 63,165 daughters derived from sires carrying homozygous p.Gly105[13] were affected (Table 2). Fisher's exact test (Agresti, 1992) indicated a difference ($P = 0.004$) between these 2 groups. Consistent with several reports that the incidence of mastitis in cows increases with age (Braund and Schultz, 1963; Batra et al., 1977; Gonyon et al., 1982), the percentage of cows affected in both heterozygous p.Gly105[12] and homozygous p.Gly105[13] daughters increased with age. At almost every age, however, the proportion of affected heterozygous p.Gly105[12] daughters was significantly lower than that of affected homozygous p.Gly105[13] daughters (Table 2). These findings confirmed that FEZL affects the incidence of mastitis as well as SCS.

Escherichia coli is very common in the dairy cow environment, and the severity of mastitis caused by this bacterium is determined mainly by cow factors rather than by *E. coli* pathogenicity (Burvenich et al., 2003). Lipopolysaccharides from *E. coli* induced *FEZL* expression in bovine mammary epithelial cells (Sugimoto et al., 2006). To examine whether the *FEZL* genotype affects the incidence of *E. coli*-caused mastitis, we com-