Effects of the Signal Transducer and Activator of Transcription 1 (*STAT1*) Gene on Milk Production Traits in Holstein Dairy Cattle

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ABSTRACT

A functional candidate gene approach was used to search for genes affecting milk production traits in Holstein dairy cattle. Signal transducer and activator of transcription 1 (*STAT1*) was chosen because of its involvement in the development of the mammary gland. Using the pooled genomic DNA sequencing approach, we identified a single nucleotide polymorphism. Genomic DNA was extracted from 1,292 sons obtained from the Cooperative Dairy DNA Repository and from 715 blood samples of daughters of 12 bulls obtained from the University of Wisconsin resource population. Daughter yield deviation data for the sons and yield deviation for the daughters were obtained for milk production traits from the USDA Animal Improvement Programs Laboratory. For the Repository population, allele C was associated with significant increases in milk fat and protein percentages. For the University of Wisconsin population, genotypes CC and CT were associated with significant increases in milk, fat, and protein yields. Results from this study are consistent with previous studies on the role of *STAT1* in regulating the transcription of genes involved in milk protein synthesis and fat metabolism. **Key words:** signal transducer and activator of transcription 1, signal transduction, quantitative trait, candidate gene

INTRODUCTION

Signal transducer and activator of transcription (*STAT*) factors are a family of cytoplasmic proteins that are activated in response to a large number of cytokines, growth factors, and hormones (Darnell, 1997). The *STAT* proteins are activated via a cascade of phosphorylation events in which Janus protein tyrosine kinases are first phosphorylated. The activated Janus protein tyrosine kinases then phosphorylate *STAT* proteins at their tyrosine residues. In turn, *STAT* detach from the receptor complex, form homo- or heterodimers, and translocate

from the cytoplasm to the nucleus, where they interact with specific promoter regions and regulate gene expression (Darnell, 1997). At present, 7 bovine *STAT* genes have been identified. *STAT1* and *STAT4* map to chromosome 2, *STAT3*, *STAT5A*, and *STAT5B* map to chromosome 19. *STAT6* maps to chromosome 5, whereas *STAT2* has not yet been mapped.

The physiological roles of *STAT* proteins were originally defined by knockout experiments in mice. *STAT4*-deficient mice gave defects in IL-12-mediated functions, including increases in IFN- γ production and signaling, cellular proliferation, and T helper 1 cell differentiation (Akira, 1999). *STAT5* consists of 2 highly related genes, which are 96% similar at the AA level. *STAT5* and *STAT3* activation may play critical roles in the regulation of growth, differentiation, and apoptosis (Bromberg, 2000). Some evidence exists that *STAT1* is involved in the development and differentiation of the mammary gland. Boutinaud and Jammes (2004) measured the expression levels of *STAT1*, *STAT3*, and *STAT5* in the mammary gland of lactating goats and found that the expression of these genes was regulated by growth hormone. Stewart et al. (1999) studied the regulation of *STAT* expression by effectors of adipocyte differentiation. They found that *STAT1*, *STAT5A*, and *STAT5B* were not exclusively regulated by individual effectors of differentiation, but their expression was tightly correlated with lipid accumulation. Studies on the expression of *STAT* in different tissues and at different developmental stages have shown that *STAT1* and *STAT3* are constitutively expressed at constant levels through pregnancy, lactation, and involution, whereas *STAT4* and *STAT5* are developmentally regulated (Watson, 2001). Moreover, Watson (2001) found that *STAT1* is regulated during mammary gland development and apoptosis and that this constitutively active gene is an oncogene in the mammary gland.

The positional and functional candidate gene approaches have been applied to different genes in dairy cattle. The first positional cloning of a QTL in cattle—the *DGAT1* gene that is associated with milk fat yield—was reported by Grisart et al. (2002). Recently, Cohen-Zinder et al. (2005) identified a missense mutation in the bovine *ABCG2* gene that affects milk yield and com-

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position in Holstein cattle. The osteopontin gene has been reported to be associated with milk protein percentage in 2 different studies (Leonard et al., 2005; Schnabel et al., 2005).

The bovine *STAT1* maps to chromosome 2 at interval 60 to 63 cM (Band et al., 2000). Whole-genome scans have shown significant associations between production traits and microsatellite markers in the vicinity of *STAT1* (Mosig et al., 2001; Ashwell et al., 2004; Ron et al., 2004). These QTL studies, along with the studies on the function, involvement, and expression of *STAT1* in the mammary gland, prompted us to investigate the effects of this gene on production traits in dairy cattle.

MATERIALS AND METHODS

Populations and Phenotypic Data

Semen samples from 29 Holstein sires and their 1,292 sons (average of 46 sons per sire) were obtained from the Cooperative Dairy DNA Repository (CDDR), which is maintained by the USDA Bovine Functional Genomics Laboratory (Beltsville, MD). In addition, 762 blood samples were obtained from the University of Wisconsin daughter design resource population (henceforth: UW resource population). This population was created to search for genetic markers in association with susceptibility to paratuberculosis. The 12 sire families of this population were chosen from a large number of candidate bulls with large numbers of daughters in production in 2000. Criteria for the final selection of the 12 bulls included large numbers of daughters in production and relatively low pedigree relationships among the chosen bulls to more broadly sample the chromosomes of the US Holstein population. Blood samples from the bulls' daughters were collected through cooperation with commercial dairy producers throughout the United States from January 2001 to July 2003. Daughter yield deviation (DYD) for sons in the CDDR and yield deviation data for daughters in the UW resource populations were obtained for milk, protein, and fat yields (kg), protein and fat percentages, and SCS from the USDA Animal Improvement Programs Laboratory (Beltsville, MD). Summary statistics of these data from both resource populations are given in Table 1.

Polymorphism Detection and Genotyping

Genomic DNA was extracted from semen samples by standard methods using proteinase K and phenol-chloroform and from blood samples using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Piscataway, NJ). The DNA concentration was measured using a spectrophotometer (Ultraspec 2100; Amersham Biosciences). To detect single nucleotide polymorphisms

Table 1. Means and standard deviations of daughter yield deviations (DYD) of sons in the Cooperative Dairy DNA Repository (CDDR) and of yield deviations (YD) of cows in the University of Wisconsin (UW) resource population for the production traits

Trait	CDDR ¹	
	Mean	SD
DYD milk, kg	561.2	765.8
DYD fat, kg	18.05	24.49
DYD protein, kg	21.37	20.69
DYD fat percentage	-0.007	0.104
DYD protein percentage	0.022	0.049
Trait	UW resource population	
	Mean	SD
Milk YD	1,092.6	1,815.1
Fat YD	29.97	68.17
Protein YD	29.86	48.73

¹The average reliabilities of CDDR sons were 0.86 for all traits.

(SNP) in the bovine expressed sequence tag (EST) corresponding to the *STAT1* gene (GenBank accession number AW289395), DNA pools were constructed from 220 bovine samples and amplified with the primers STATF (positions 12 to 31): 5'-GCCTCAAGTTTGCCAGTGGC-3' and STATR (positions 325 to 306): 5'-GGCTCCCTTG ATAGAACTGT-3'. Amplification was performed in a 25- μ L reaction volume, which included 50 ng of genomic DNA, 50 ng of each primer, 200 μ M each dNTP, 2.5 μ L of 10 $\times$ PCR buffer (Promega, Madison, WI), and 0.3 U of *Taq* DNA polymerase (Promega). The temperature cycles were as follows: 95°C for 5 min, followed by 30 cycles of 94°C for 45 s, touchdown annealing from 65 to 50°C for 45 s (-2°C/cycle), 72°C for 45 s, and a final extension at 72°C for 7 min. Polymerase chain reaction products of the pooled DNA samples were sequenced and SNP were identified by visually inspecting sequence traces. For individual genotyping, the primers STATF and STATR were used to amplify 50 ng of genomic DNA and the PCR products were digested with the restriction enzyme *PagI*, which distinguishes alleles C and T of the SNP. The digestion products were electrophoresed on a 1.5% agarose gel; the T allele was indicated by a band of 314 bp and the C allele was indicated by 2 bands of 201 and 113 bp.

Statistical Analysis

The CDDR Population. A weighted least squares analysis was used to study the effects of *STAT1* variants on milk production traits in the CDDR population. The model was

$$y_{ij} = \mu + \text{sire}_i + \beta x_{ij} + e_{ij} \quad [1]$$

where y_{ij} is the DYD of the trait for son j of sire i , $sire_i$ is the fixed effect of sire i , β is the regression coefficient representing one-half the allele substitution effect ($\alpha/2$); x_{ij} is the number of C alleles (0, 1, or 2) for the j th son of sire i , and e_{ij} is the residual. Reliabilities of the sons' DYD were incorporated as weights in the model to obtain weighted least squares estimates for the allele substitution effects.

The UW Resource Population. For the daughters in the UW resource population, allelic and genotypic effects of *STAT1* were analyzed using both Models [1] and [2]. Model [2] was

$$y_{ijkl} = \mu + \text{genotype}_i + \text{sire}_j + \text{Map}_K + e_{ijkl} \quad [2]$$

where y_{ijkl} is the yield deviation (milk, fat, protein) of daughter l of sire j , μ is the mean, genotype_i is the effect of daughter genotype i (CC, CT, TT), sire_j is the sire j effect, Map_K is *Mycobacterium avium* ssp. *paratuberculosis* infection status (noninfected = 0, infected = 1), and e_{ijkl} is the residual. Two diagnostic tests, ELISA antibody and fecal culture for the pathogen, were done to determine infection status. Infection status was positive for cows that were positive for either test. The additive genetic effect was estimated as one-half the difference between the 2 homozygous genotypes. Similarly, the dominance genetic effect was estimated as the difference between the average of the 2 homozygous genotypes and the heterozygous genotype. The TT genotype was set as the baseline for estimating the genotypic effects.

RESULTS

Using the pooled genomic DNA sequencing approach, we identified an SNP (C/T) at position 213 in the EST corresponding to *STAT1*. The complete sequence of bovine *STAT1* has not yet been reported. However, the basic local alignment search tool (BLAST) revealed 100% similarity between this EST and the predicted sequence of the 3' untranslated region (UTR) of the *STAT1* gene (GenBank accession number XM_872927) recently released by the Baylor College of Medicine's Human Genome Sequencing Center. Thus, SNP C/T corresponded to position 3141 in the 3' UTR of the predicted sequence of *STAT1*.

Frequency of the C allele was 0.66 in both the CDDR and the UW resource populations. Genotype frequencies were as expected for the Hardy-Weinberg equilibrium. Table 2 shows the estimated regression coefficients on the number of copies of the C allele (one-half the allele substitution effects, $\alpha/2$) in the CDDR and the UW resource populations. For the CDDR population, a significant effect of *STAT1* variants on milk fat percentage ($P = 0.033$) and milk protein percentage ($P = 0.042$) was

Table 2. Estimates of the allele substitution effects (replacing T with C) and standard errors for milk production traits in the Cooperative Dairy DNA Repository (CDDR) and the University of Wisconsin (UW) resource population

Trait	CDDR population, $\alpha/2 \pm \text{SE}$	UW resource population, $\alpha/2 \pm \text{SE}$
Milk yield	-19.50 ± 29.65	94.10 ± 66.88
Fat yield	1.75 ± 1.07	3.79 ± 2.48
Fat percentage	$0.010 \pm 0.004^*$	NA ²
Protein yield	0.510 ± 0.730	$3.57 \pm 1.80^*$
Protein percentage	$0.004 \pm 0.002^*$	NA
SCS	$0.019 \pm 0.010^\dagger$	-0.041 ± 0.039

¹Estimated regression coefficient β representing half the allele substitution effect.

²NA = data not available.

[†] $P < 0.10$; * $P < 0.05$.

found in across-family analysis (Table 2). The estimated increase in milk fat percentage of the C allele was 0.01%. The C allele was also associated with an increase in milk protein percentage compared with the T allele (Table 2). The C allele seems to be associated with an increase in SCS compared with the T allele, with borderline significance (Table 2). For the UW resource population, the C allele was associated with an increase in milk protein yield ($P = 0.048$) compared with the T allele.

Table 3 shows the estimates of the genotype effects for the yield traits in the UW resource population. Genotypes CC and CT were associated with significant increases in milk, fat, and protein yields compared with genotype TT. Additive gene action, one-half the contrast between the 2 homozygous genotypes, was significant for milk, fat, and protein yields (Table 3). Dominant gene action was tested as the average of the 2 homozygous classes compared with the heterozygous genotype. The dominance effect was significant for fat yield ($P = 0.055$). The hypothesis tests of differences between the CT and CC genotypes (P -values being 0.780, 0.941, and 0.298 for milk, fat, and protein yields, respectively) suggested possible complete dominance of C over T. Although the dominance effect was not significant for milk and protein yields, the degree of dominance, taken as the ratio of the dominance effect to the additive effect, was 0.84 and 0.51, respectively (Table 3). However, with a small number of TT genotype daughters, the estimates of dominance deviation had large coefficients of variation (Table 3). Nevertheless, in both the CDDR and UW resource populations, allele C and genotype CC, respectively, were associated with significant increases in milk composition traits.

DISCUSSION

This study reports the association between *STAT1* variants and milk production traits in Holstein dairy

Table 3. Estimates of the genotypic effects ($\pm$ SE) as a deviation from the genotype (TT),¹ genetic effects ($\pm$ SE), and dominance deviations for milk production traits in the University of Wisconsin resource population

Effect	Milk yield $\pm$ SE	Fat yield $\pm$ SE	Protein yield $\pm$ SE
Genotypic effect			
CC (n = 298)	515.7 $\pm$ 250.2*	21.90 $\pm$ 9.30*	17.05 $\pm$ 6.74*
CT (n = 341)	475.6 $\pm$ 229.1*	21.49 $\pm$ 8.52*	12.86 $\pm$ 6.19*
Genetic effect			
Additive	257.8 $\pm$ 125.1*	10.95 $\pm$ 4.65*	8.53 $\pm$ 3.37*
Dominance	217.8 $\pm$ 147.8	10.54 $\pm$ 5.49†	4.33 $\pm$ 3.99
Degree of dominance ²	0.84	0.96	0.51

¹Number of daughters with the TT genotype was 76.

²Ratio of the dominance effect to the additive effect.

† $P < 0.10$; * $P < 0.05$.

cattle. The *STAT1* gene was chosen because of its expression and involvement in the development of the mammary gland and because a QTL for milk yield and composition traits had previously been shown in the region of the gene.

The pooled DNA sequencing approach has been used in several studies both for polymorphism identification and for estimation of allele frequencies in pooled DNA or RNA samples. Kwok et al. (1994) reported the identification of SNP with >20% allele frequency for the minor allele. In previous studies, pooled DNA and RNA samples were used to identify SNP in coding and noncoding regions in candidate genes affecting milk production traits (Khatib et al., 2005; Khatib et al., 2006). In this study, using the pooled DNA sequence-based method, one SNP (C/T) was detected in a total length of 610 bp. This SNP was significantly associated with an increase in milk yield and composition traits in both the CDDR and UW resource populations.

For the CDDR population, using the allele substitution model, the C allele was associated with an increase in milk fat percentage and milk protein percentage. The correlation between these 2 traits was 0.57 (Khatib et al., 2005). When the same statistical model was used in the UW resource population, allele C was associated with an increase in milk protein yield. No data on percentage traits were available in the UW resource population. Moreover, in this population, the estimates of the genotypic effects (CC and CT vs. TT genotypes) were consistent with the estimates of the allele substitution effects, so there was no contradiction between the effects observed in the CDDR and UW resource populations. The significant association between genotypes CC and CT and yield traits in the UW resource population indicates a large biological effect associated with the contrast among these genotypes. The small effects obtained in the allele substitution model compared with the large effects obtained in the genotypic model were due to the high frequency of allele C in the population. Thus, when the allele substitution model was used in both popula-

tions and the modes of gene action were analyzed in the UW resource population, *STAT1* variants were associated with an increase in milk yield and composition traits.

Results from this study are consistent with other studies that have shown significant association of genetic markers with milk composition traits in the region of *STAT1* (interval 60 to 63 cM). Mosig et al. (2001) reported a putative QTL affecting milk protein percentage in linkage with microsatellite marker BMS1126 at position 61.7 cM from the centromere. In addition, Ashwell et al. (2004) reported a QTL affecting milk fat percentage in linkage with microsatellites ETH121 and BM4440 at the interval 38.0 to 60.3 cM. Also, Ron et al. (2004) reported a QTL affecting milk protein percentage at the interval 61.7 to 70 cM from the centromere.

The observed associations of bovine *STAT1* with milk production traits were not surprising for the following reasons: 1) The expression of *STAT1* is under control of the hormone prolactin. Following binding of prolactin to its receptor, a cascade of events is initiated that leads to activation of the STAT1, STAT3, and STAT5 proteins, which in turn regulate the transcription of genes involved in secretion of milk proteins and components (Tucker, 2000; Bole-Feysot et al., 2005). 2) There is evidence that the *STAT* genes might be important for the regulation of fat metabolism and milk protein synthesis, probably through the prolactin signal transduction pathway operating in the mammary gland (Mao et al., 2002). Our results show that *STAT1* was associated with milk fat and protein yields and percentages. 3) Interferons regulate cellular antiviral, antiproliferative, and immunological responses. *STAT1* has been shown to be essential for cell growth suppression in response to IFN- γ (Akira, 1999). Moreover, *Stat1*-deficient mice were reported to be highly sensitive to infection by pathogens and to develop tumors more frequently than normal mice (Akira, 1999; Watson, 2001). These studies indicate that *STAT1* may have roles in the immune response. Results from this study on the effect of *STAT1* on somatic cells

in milk, an indicator for mammary gland health in cows, are consistent with reported functions of this gene in the immune response of the human and mouse.

Recently, we used positional and functional candidate gene analysis to search for candidate genes affecting milk production traits. A significant association was found between different haplotypes of the protease inhibitor gene and several production traits in Holstein dairy cattle, including milk yield, milk fat yield, and SCS (Khatib et al., 2005). Using this approach, we also analyzed the oxidized low-density lipoprotein receptor (*OLR1*) as a candidate gene affecting milk production traits (Khatib et al., 2006). One SNP in the 3' UTR was associated with significant increases in milk fat yield and milk fat percentage.

The complete sequence of the bovine *STAT1* has not yet been validated by the Baylor College of Medicine's Human Genome Sequencing Center. Thus, it would be important to characterize the genomic structure of the gene, identify more SNP, and search for causative polymorphism(s) associated with production traits. However, whether the C/T SNP in the 3' UTR of the gene is the actual causative mutation affecting milk composition traits, *STAT1* is the causative gene, or the SNP is in linkage disequilibrium with other gene(s) affecting these traits, we consider this *STAT1* SNP a strong candidate for marker-assisted selection programs in dairy cattle. Marker-assisted selection can increase allele frequencies of the favorable alleles within a relatively few generations of selection, as has been demonstrated for the calpain and calpastatin genes in beef cattle (Pollak, 2005).

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