The Association of Bovine *PPARGC1A* and *OPN* Genes with Milk Composition in Two Independent Holstein Cattle Populations

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ABSTRACT

Many studies have reported quantitative trait loci on chromosome 6 that affect milk production traits in dairy cattle. Osteopontin (*OPN*) and peroxisome proliferator activated receptor gamma coactivator 1 alpha (*PPARGC1A*) are located in the middle of chromosome 6 about 6 Mb apart, which is approximately 12 cM. The objective of this study was to investigate the association of *OPN* and *PPARGC1A* variants with milk production traits in 2 independent Holstein cattle populations: the University of Wisconsin (UW) daughter design and the Cooperative Dairy DNA Repository (CDDR) granddaughter design resource populations. For *OPN*, 891 cows from the UW resource population were genotyped for the C/T polymorphism reported previously in the CDDR population. Additive effects were significant for fat percentage, protein percentage, and fat yield in the UW resource population. These results are consistent with previous studies that have shown significant association of *OPN* variants with milk composition traits. The association between *PPARGC1A* variants was investigated in UW and CDDR resource populations using 2 single nucleotide polymorphisms. For the UW resource population, additive effects were significantly increased for protein percentage and decreased for milk yield. Dominance effects were not significant for any of the examined traits. For the CDDR population, *PPARGC1A* was associated with a significant increase in milk protein percentage and in SCS. Thus, in UW and CDDR populations, *PPARGC1A* was associated with a significant increase in milk protein percentage in contrast to association results previously reported for the German Holstein population.

Key words: *OPN*, *PPARGC1A*, candidate gene, single nucleotide polymorphism

INTRODUCTION

In dairy cattle and other livestock species, there is increasing interest in using a positional candidate gene approach for the identification of the actual genes that control economically important traits. At present, candidate quantitative trait genes are chosen for association studies because of previous linkage mapping studies and comparative biological functions in the same or other species (Rothschild and Soller, 1997). Many studies have reported QTL on multiple chromosomes that affect milk production and health traits in dairy cattle. Chromosome 6 contains the highest number of reported QTL (Khatkar et al., 2004). Meta-analysis of these QTL studies suggests that one QTL affecting milk, fat, and protein yield and fat and protein percentages is located around 50 cM, near microsatellite BM143 (Khatkar et al., 2004). There was also evidence for a second QTL affecting milk yield and protein percentage around 87 cM. For the QTL in the middle of the chromosome, 8 studies reported QTL affecting milk yield, 11 reported QTL affecting protein percentage, 5 reported QTL affecting protein yield, and 7 reported QTL affecting fat percentage (Khatkar et al., 2004).

Ron et al. (2001) localized a QTL affecting protein percentage to a confidence interval of 4 cM in the region near 50 cM on chromosome 6. Recently, Olsen et al. (2005) positioned a QTL affecting milk production traits at an interval of 420 kb between the genes *ABCG2* and *LAP3* on bovine chromosome 6. This narrow region harbors only 6 genes including the osteopontin (*OPN*) gene. Based on the aforementioned QTL studies, several groups investigated possible associations between genes in this region and milk production traits in dairy cattle. Weikard et al. (2005) reported significant association between a single nucleotide polymorphism (SNP) in intron 9 of the peroxisome proliferator activated receptor gamma coactivator 1 alpha (*PPARGC1A*) gene (close to microsatellite BM143) and milk fat yield in the German Holstein population. Leonard et al. (2005) and Schnabel et al. (2005) reported an association between *OPN* and milk protein percentage in the North American Holstein population. Furthermore, Schnabel

Received December 4, 2006.

Accepted February 4, 2007.

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et al. (2005) presented several lines of evidence for a quantitative trait nucleotide, located upstream of *OPN* promoter region, causing variation in milk protein percentage. On the other hand, Cohen-Zinder et al. (2005) reported the identification of a causative mutation in *ABCG2* affecting milk yield and composition and excluded the *OPN* mutation reported in the Schnabel study from causality. The results for these conflicting candidate genes for the QTL in the middle of chromosome 6 necessitate additional populations to be tested for these genes (de Koning, 2006). In this study, the association of *OPN* variants with milk composition was investigated in an independent Holstein population for the validation of results previously obtained in a different granddaughter design Holstein population. In addition, the association between *PPARGC1A* variants, also located in the middle of chromosome 6, and milk production traits was also investigated in the University of Wisconsin (UW) daughter design and in the Cooperative Dairy DNA Repository (CDDR) granddaughter design resource populations. The *OPN* and *PPARGC1A* are about 6 Mb apart, which is about 12 cM for this region of chromosome 6 (<http://www.ncbi.nlm.nih.gov/mapview>).

MATERIALS AND METHODS

Populations and Data

Blood samples ($n = 931$) were obtained from the UW resource population. This population was originally created to search for QTL in association with susceptibility to paratuberculosis (Gonda et al., 2006). Cows were sampled from 300 herds scattered throughout the United States. The 12 sire families of this population were chosen because they included large numbers of daughters in production and because of relatively low pedigree relationships among the bulls. Of 66 possible pairings of sires, genetic relationships were 0.25 for 1 pair of sires, 0.125 for 7 pairs, 0.0625 for 13 pairs, and lower for the remaining 45 pairs (Gonda et al., 2006).

In addition, semen samples from 12 Holstein sires and their 581 sons were obtained from the CDDR, which is maintained by the USDA Bovine Functional Genomics Laboratory (Beltsville, MD). Predicted transmitting ability data for sons in the CDDR and yield deviation 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).

OPN and *PPARGC1A* SNP

It was previously shown that a C/T polymorphism at position 8514 of *OPN* (GenBank accession number

NW_255516) is associated with milk protein and fat percentages in 1,362 bulls obtained from 28 sire families from the CDDR population (Leonard et al., 2005). To validate this association, 891 cows from the UW resource population were genotyped for this polymorphism. The association of *PPARGC1A* SNP with milk production was investigated in the UW and the CDDR resource populations using 2 SNP reported by Weikard et al. (2005); SNP C/T at position 1892 and SNP A/C at position 3359. The SNP 1892 was genotyped only for the UW resource population; SNP 3359 was genotyped in both populations.

SNP Genotyping

Genomic DNA was extracted from blood samples using GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Piscataway, NJ) and from semen samples by standard methods using proteinase K and phenol/chloroform. Primer sequences and amplification conditions for *OPN* were described in Leonard et al. (2005). Primers 1892F 5'-CATAGCCGGCGCCCCAGGTAAGATGCACGTTGGC-3' and 1892R 5'-CTGGTACTCCTCGTAGCTGTC-3' were used to amplify 195 bp in intron 9 to genotype SNP C/T at position 1892 of *PPARGC1A*. Primers 3359F 5'-GCGAGCACGGTGTTCAT TACTAAGGAGAGTTGGCTAG-3' and 3359R 5'-GTTGTGTTGCACTCAATGGAC-3' were used to amplify 357 bp in the 3' untranslated region (3' UTR) of *PPARGC1A* to genotype SNP A/C at position 3359. Primers 1892R and 3359R were used by Weikard et al. (2005). Amplification was performed in a 15- μ L reaction volume, which included 25 ng of genomic DNA, 25 ng of each primer, 100 μ M of each dNTP, 1.5 μ L of 10 $\times$ PCR buffer (Promega, Madison, WI), and 0.2 U of Taq DNA polymerase (Promega). The temperature cycles were as follows: 95°C for 5 min, followed by 32 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 7 min. For genotyping of SNP C/T at position 1892, PCR products were digested with the restriction enzyme *Hae*III that distinguishes alleles C and T of the SNP. For genotyping of SNP A/C at position 3359, PCR products were digested with the restriction enzyme *Nhe*I that distinguishes alleles A and C. The digestion products were electrophoresed on a 2.5% agarose gel.

Statistical Analysis

The association of *OPN* and *PPARGC1A* SNP with production traits was studied in 2 Holstein populations (CDDR and UW). The CDDR phenotypic data referred to bulls' PTA data for milk, protein and fat yield, protein and fat percentages, and SCS. The UW resource population data was composed of yield deviations of cows for the same traits.

CDDR Population. Associations between *OPN* variants and the phenotypic traits recorded in the CDDR population were published by Leonard et al. (2005). Therefore, in the present work only the *PPARGC1A* locus was considered in this population. Because the CDDR data are PTA, effects of an additional positive allele in the sons of a sire are necessarily additive. Hence, for this data set an allele substitution model having the following structure was used for the analysis of each trait:

$$y_{ij} = \mu + s_i + \beta x_{ij} + \varepsilon_{ij},$$

where y_{ij} is the PTA value relative to son j of sire i , μ is a general constant (intercept), s_i is the fixed effect of sire i , β is the regression coefficient representing half of the allele substitution effect ($\alpha/2$), x_{ij} is the number of A alleles (0, 1, or 2) at the *PPARGC1A* locus of son j of sire i , and ε_{ij} is a residual term. Reliabilities of the sons' PTA were incorporated as weights in the model to obtain weighted least squares estimates of the allele substitution effects.

UW Resource Population. Because the UW resource data are individual cow records, allele effects are not necessarily additive. Hence, data relative to each trait in the UW resource population were analyzed using the following fixed effects model:

$$y_{ijkl} = \mu + s_i + d_{ij}\tau + o_k + p_l + \varepsilon_{ijkl},$$

where y_{ijkl} represents the yield deviation of daughter j of sire i ; τ is an effect associated with *M. paratuberculosis* infection status; d_{ij} is an indicator variable assuming the values 0 and 1 for noninfected and infected cows, respectively; o_k is the effect of the *OPN* genotype ($k = \text{CC, CT, or TT}$); and p_l is the effect of the *PPARGC1A* genotype ($l = \text{AA, AC, or CC}$). The remaining terms in the model are as previously defined. Both *OPN* and *PPARGC1A* were analyzed in the same model to control for variation introduced by each locus. All interaction terms were included in the error term. This was not done for CDDR data because only the *PPARGC1A* locus was considered in this population at this time. Additive genetic effect of each locus was estimated as half of the difference between the 2 homozygous groups, as $(\hat{o}_{\text{CC}} - \hat{o}_{\text{TT}})/2$ for *OPN* and $(\hat{p}_{\text{AA}} - \hat{p}_{\text{CC}})/2$ for *PPARGC1A*. The dominance effect was estimated as the difference between the heterozygous group and the average of the 2 homozygous groups in each locus. The analyses were implemented using the GLM procedure of SAS (SAS Institute, 1999).

Table 1. Estimates ($\pm$ SE) of additive and dominance effects associated with the single nucleotide polymorphism at position 8514 of the osteopontin (*OPN*) gene in the University of Wisconsin resource population

Trait	Additive effect	Dominance effect
Fat yield	9.32 ($\pm$ 3.79)*	4.23 ($\pm$ 4.55)
Fat percentage	0.0496 ($\pm$ 0.0133)***	-0.0020 ($\pm$ 0.0160)
Milk yield	-83.63 ($\pm$ 101.6)	133.0 ($\pm$ 122.0)
Protein yield	3.75 ($\pm$ 2.70)	4.79 ($\pm$ 3.24)
Protein percentage	0.0251 ($\pm$ 0.0062)***	0.0036 ($\pm$ 0.0074)
SCS	0.0085 ($\pm$ 0.0583)	0.0100 ($\pm$ 0.0698)

* $P < 0.05$; *** $P < 0.001$.

RESULTS

OPN

Frequencies of CC, CT, and TT genotypes in the UW population were 0.23, 0.51, and 0.26, respectively, and were in close accord with Hardy-Weinberg (**H-W**) expectations. Table 1 shows the estimates of the additive and dominance genotypic effects SNP C/T at position 8514 of *OPN* for milk yield and composition traits and for SCS in the UW resource population. Additive effects were significant for fat percentage ($P < 0.0001$), protein percentage ($P < 0.0001$), and fat yield ($P = 0.014$). Dominance effects were not significant for any of the examined traits.

PPARGC1A

The SNP C/T at position 1892 was genotyped only for the UW resource population, and the frequencies of genotypes CC, CT, and TT of SNP 1892 were 33.1, 65.0, and 1.9%, respectively. Genotype frequencies differed strongly and with high significance from H-W expectations. In particular, there was a marked deficiency of TT homozygotes, raising the possibility of a recessive semilethal associated with this genotype. Association tests between SNP 1892 and milk production traits in the UW resource population were not significant for any of the examined traits (data not shown). Therefore, this SNP was not tested further in the CDDR population. In contrast, the association between SNP A/C at position 3359 and milk production traits was examined in UW and CDDR populations. For the UW population, the frequencies of genotypes AA, AC, and CC at this SNP were 16.3, 50.6, and 33.1%, respectively, and for the CDDR population, the frequencies of these genotypes were 12.3, 43.0, and 44.7%, respectively. In both populations genotype frequencies were in close accord with H-W expectations. Table 2 shows estimates of additive and dominance effects of *PPARGC1A* locus in the UW population and the average allele substitution effects for production traits in the CDDR population.

Table 2. Estimates ($\pm$ SE) of additive and dominance effects associated with the single nucleotide polymorphism at location 3359 of the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*) gene in the University of Wisconsin (UW) resource population and estimates ($\pm$ SE) of the average allele substitution effect (allele A) in the CDDR¹ population

Trait	Additive effect ²	Dominance effect ²	Allele substitution ³
Fat yield	-4.18 ($\pm$ 3.85)	5.40 ($\pm$ 4.75)	-1.20 ($\pm$ 2.82)
Fat percentage	0.0189 ($\pm$ 0.0138)	0.0011 ($\pm$ 0.0171)	0.008 ($\pm$ 0.010)
Milk yield	-238.6 ($\pm$ 102.6)*	128.2 ($\pm$ 126.6)	-97.6 ($\pm$ 73.0)
Protein yield	-3.04 ($\pm$ 2.73)	6.58 ($\pm$ 3.37)	0.264 ($\pm$ 1.908)
Protein percentage	0.0185 ($\pm$ 0.0064)**	0.0107 ($\pm$ 0.0080)	0.012 ($\pm$ 0.004)*
SCS	-0.0003 ($\pm$ 0.0586)	0.0206 ($\pm$ 0.0724)	0.042 ($\pm$ 0.020)*

¹Cooperative Dairy DNA Repository.

²Estimates from yield deviations from the UW resource population.

³Estimates from PTA from the CDDR population.

* $P < 0.05$; ** $P < 0.01$.

Significant effects were found for milk yield in the UW population ($P = 0.020$); for protein percentage in UW and CDDR populations ($P = 0.004$ and 0.016 , respectively); and for SCS in the CDDR population ($P = 0.038$). Dominance effects, which were included in the model for analysis of the UW data, were not significant for any of the examined traits. Direction of additive effect (UW) and allele substitution effect (CDDR) were the same for all traits, except protein yield and SCS. In particular, the A allele was associated with significant positive effects on protein percentage in both populations. Also, there was an indication of a decrease in milk yield for this allele in both populations, although it was not significant in the CDDR population. This is not surprising given that the correlation between protein percentage and milk yield in the CDDR population was -0.40 (Khatib et al., 2005).

DISCUSSION

The results of the present study are consistent with previous studies that have shown significant association of *OPN* variants with milk composition traits (Cohen et al., 2004; Leonard et al., 2005; Schnabel et al., 2005). Cohen et al. (2004) investigated the association of 12 SNP in 10 genes, located in the region of the microsatellite BM143 in the middle of chromosome 6, and milk composition in 420 Holstein bulls. Among these genes, *OPN* showed the highest linkage disequilibrium effects on protein percentage. In addition, based on expression analysis in mammary gland in cattle (Cohen et al., 2004) and on abnormal differentiation of mammary gland in antisense transgenic *OPN* mice (Nemir et al., 2000), Cohen et al. (2004) concluded that *OPN* was the key candidate gene affecting milk protein percentage. Conversely, in a subsequent study, Cohen-Zinder et al. (2005) sequenced bulls with known QTL for SNP OPN3907, reported by Schnabel et al. (2005) to be the quantitative trait nucleotide, and found that

this SNP region was hyper variable and that 17 of 18 bulls were heterozygous for at least one polymorphism. Accordingly, they concluded that *ABCG2* but not *OPN* is the quantitative trait gene affecting milk composition in the middle of chromosome 6 (Cohen-Zinder et al., 2005).

The *PPARGC1A* gene was chosen for association test with milk fat traits because of several QTL studies that showed effects of *PPARGC1A* region on milk production and because of possible involvement of this gene in fat metabolism (Weikard et al., 2005). The authors reported a significant association between SNP 1892 in intron 9 and milk yield and milk fat traits in 434 bulls from the German Holstein population. The SNP 3359 in the 3' UTR showed some trend of association with fat traits, but it did not reach the significance level of 5%, so the authors attributed this association to the linkage disequilibrium between the 2 SNP.

In contrast to Weikard et al. (2005), in the current study a significant association was found between SNP 3359 and protein percentage and milk yield, whereas SNP 1892 did not show significant association with any of the examined traits. These conflicting findings between results from this study and results from Weikard et al. (2005) could be explained by the 2 different genetic backgrounds of the studied populations or by the different number of samples included in each study. In the current study, *PPARGC1A* was genotyped in 2 independent populations with more than 1,400 individuals, whereas in the study by Weikard et al. (2005) 434 bulls were genotyped. Also, different associations of different SNP in the same gene in 2 or more populations could be a result of linkage disequilibrium of these SNP with the causative mutation in the same gene or in other genes. The SNP showing significant association with milk production traits would provide an excellent opportunity for marker-assisted selection (MAS) programs in dairy cattle. The aim of MAS is to substitute selection at the DNA level for selection on the basis of

phenotype (Soller, 1994). Also, MAS can increase allele frequencies of the favorable alleles, as has been shown for the calpain and calpastatin genes in beef cattle (Pollak, 2005). However, for MAS to be efficient in genetic improvement programs, it is assumed that the effects of genes used in MAS are known and are consistent in different genetic backgrounds and environments (Dekkers and Hospital, 2002). The inconsistent association results between our data and Weikard's necessitate further investigation prior to applications in MAS programs.

ACKNOWLEDGMENTS

This research was supported by start-up funding from the University of Wisconsin–Madison and by The Draper Technology Innovation Fund from Graduate School, the University of Wisconsin–Madison. We thank George E. Shook for providing blood samples from the UW resource population. We thank the staff and administration of the USDA Bovine Functional Genomics Laboratory for providing semen samples from the CDDR population.

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