Single Nucleotide Polymorphisms in Candidate Genes Related to Daughter Pregnancy Rate in Holstein Cows

*M.S. Ortega**, A.C. Denicol*, D.J. Null†, J.B. Cole† and P.J. Hansen*

* Department of Animal Sciences, University of Florida, Gainesville, Florida, USA; † Animal Improvement Programs Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, USA

ABSTRACT: Previously, a candidate gene approach identified 40 SNPs associated with daughter pregnancy rate (DPR) in dairy bulls. We evaluated 39 of these SNPs for relationship to DPR in a separate population of Holstein cows grouped on their predicted transmitting ability for DPR: ≤ -1 (n=1266) and ≥ 1.5 (n=1071). Genotyping was by Sequenom MassARRAY®. Of the 39 SNPs, 30 were related to DPR in the same direction as previously reported, with significance for 18. SNPs that explained the greatest proportion of variation in DPR were *COQ9* (3.0%), *HSD17B12* (1.6%), *APBB1* (1.3%), *FUT1* (1.2%), and *C7H19orf60* (1.2%). Overall, the 39 SNPs explained 17.6% of the variation in DPR. Results indicate that a large proportion of candidate gene SNPs previously related to DPR are predictive in a separate population. Use of these SNPs may increase reliability of genomic estimates of DPR.

Key words: daughter pregnancy rate; fertility; dairy cattle.

Introduction

Daughter pregnancy rate (DPR) is a trait widely used in the United States and elsewhere to estimate genetic merit for reproductive ability. Heritability of this trait is very low (0.04; Van Raden et al. 2004) so that progress in selection using traditional breeding programs is slow. Moreover, genome wide association analysis has been less effective at increasing reliability of genetic estimates of DPR than for more heritable traits (Wiggans et al. 2011). Given that most single nucleotide polymorphisms (SNPs) on bovine genotyping arrays are not in the coding regions of genes (Michelizzi et al. 2011), identification of causative SNPs affecting reproductive function might be useful for improving reliability of genotyping arrays for DPR.

Recently, Cochran et al. (2013) identified SNPs in 40 genes related to DPR in dairy bulls. SNPs were identified based on a known role in reproductive processes, previous reports of a relationship to DPR, or because the gene is differentially expressed between physiological conditions in one or more tissue associated with reproduction. Most SNPs were missense mutations in the coding region of the gene.

The purpose of the present study was to evaluate the relationship of these SNP with the genetic value for DPR in a separate population of Holstein cows.

MATERIALS AND METHODS

Sample population Cows were chosen based on their predicted transmitting ability (PTA) and reliability for DPR. The values for PTA and reliability were obtained from the national genetic evaluation system. Cows were selected to have a high (≥1.5) or low PTA for DPR (≤ -1.0). The minimum reliability for inclusion was 0.25. The regressed PTA value for DPR ranged from -1 to 4, and reliabilities ranged from 0.25 to 0.77. Cows located on a total of 11 farms were sampled with 1071 cows in the high DPR group and 1266 in the low DPR group. All the animals had at least a lactation completed at the moment of the sampling (range: 1-7 lactations).

Genotyping Blood samples were collected and sent to GeneSeek Inc. (Lincoln, NE, USA) for DNA isolation and genotyping. Genotype for the 40 SNPs previously reported as being related to DPR by Cochran et al. (2013) was determined using the Sequenom MassARRAY® system (Abel et al. 2006). The average call rate was 96%. Genotype for a random sample of 10 SNPs was determined in duplicate for each animal. Agreement between duplicates was >85%. When the genotype did not match between samples, both genotypes were deleted and treated as no call.

The assay for one SNP (*MARVELD1*) was not considered acceptable (all animals were called as homozygous for one allele) and these data were not considered in statistical analysis.

Statistical analyses Allele frequency was determined using the FREQ procedure of SAS (V9.3; SAS Institute Inc. Cary, NC). SNP effects were estimated for the deregressed values of DPR in two analyses. First, genotype was considered a continuous variable to determine the allele substitution effect (the additive effect of the number of copies of the major allele; least-squares means represent values for 0, 1 and 2 copies of the major allele). In the second, genotype was considered a categorical variable, and an orthogonal contrast was used to estimate dominance effects. SNPs in which the additive or dominance effect was $P < 0.05$ were considered significant. Additionally, a multiple regression analysis was performed using the PROC GLM procedure of SAS using the 39 SNPs as explanatory variables for DPR.

RESULTS AND DISCUSSION

For 37 of 39 SNPs evaluated, the major allele was the same as the major allele reported by Cochran et al. (2013). The exception was for *BSP3* and *PARM1*.

Data on SNP effects on DPR were interpreted in two ways – whether the additive or dominance effect had a significant association with DPR and whether the favorable genotype was the same or opposite from that found by Cochran et al. (2013).

There were significant relationships with DPR for 23 SNPs, with 19 having an additive effect (*COQ9,*

HSD17B12, APBB1, FUT1, C7H19or60, DSC2, ACAT2, MS4A8B, CAST, BSP3, PCCB, OCLN, RABEP2, CACNA1D, HSD17B7, MON1B, TDRKH and GPLD1), 2 having a dominance effect (*LBD3* and *TSHB*) and 2 (*C7H19or60* and *DSC2*) having additive and dominance effects. Overall, the relationship between genotype and DPR was in the same direction as that found by Cochran et al. (2013) for 31 of 39 SNPs.

Table 1. Allele substitution effects on daughter pregnancy rate for ten SNPs explaining the greater variation in daughter pregnancy rate

Gene	Copies of major allele[§]			R^2	*P* [¥]	
	0	1	2		A	D
COQ9	4.49 (0.60)	0.68 (0.43)	-2.45 (0.57)	0.030	<0.0001	0.573
HSD17B12	4.08 (0.71)	0.77 (0.43)	-1.27 (0.53)	0.016	<0.0001	0.311
APBB1	-2.99 (1.41)	-1.08 (0.51)	2.00 (0.38)	0.013	0.0007	0.513
FUT1	-5.17 (1.59)	-0.72 (0.53)	1.77 (0.37)	0.012	<0.0001	0.320
C7H19orf60	-0.32 (0.97)	-1.06 (0.49)	2.21 (0.43)	0.012	0.0172	0.005
DSC2	2.01 (0.71)	1.80 (0.43)	-1.50 (0.54)	0.011	<0.0001	0.013
ACAT2	3.82 (0.78)	1.16 (0.45)	-0.75 (0.47)	0.011	<0.0001	0.567
MS4A8B	-2.14 (1.19)	-0.74 (0.50)	2.06 (0.40)	0.011	0.0008	0.380
CAST	-1.61 (0.65)	0.48 (0.43)	2.46 (0.53)	0.010	<0.0001	0.918
BSP3	-2.10 (0.99)	-0.08 (0.47)	2.01 (0.43)	0.008	0.0002	0.956

[§]Least-squares means (SEM).
[¥]A.: Additive or D: dominance.

Allele substitution effects for the 10 SNPs that explained the greatest variation in DPR are shown in Table 1. Of these SNPs, 8 genotype effects were in the same direction found by Cochran et al. (2013) while 2 (*APBB1* and *DSC2*) were in the opposite direction. These 10 genes had been identified by Cochran et al. (2013) for various reasons. *COQ9* was chosen because expression in embryos was increased by IGF1 (Bonilla et al. 2011), *FUT1* was differentially regulated between embryos produced by artificial insemination versus superovulation (Gad et al. 2011) and *DSC2* was upregulated following cryopreservation and differentially expressed between embryos produced via different methods (Kuzmany et al. 2011). *APBB1* and *C7H19orf60* are genes that were differentially regulated in the endometrium of lactating vs non-lactating cows (Cerri et al. 2012) while endometrial expression of *BSP3, ACAT2,* and *MS4A8B* was related to embryo survival (Beltman et al. 2010; Salilew-Wondim et al. 2010). *CAST* had been reported to be related to DPR in a previous study (Garcia et al., 2006) and *HSD17B12* is a well-known gene involved in reproduction that participates in the conversion of estrone to estradiol (Luu-The et al. 2006).

Using multiple regression analysis, the 39 SNPs explained 17.1% of the variation in DPR. This compares to an R^2 of 0.53 for the top 100 most significant SNP for DPR on the Bovine SNP50 BeadChip (Cole et al., 2011). Previous results indicate that several of the 39 SNPs examined here are not physically close to SNPs on the SNP50 Bead Chip (Cochran et al., 2013). Further research will be conducted to determine effectiveness of incorporation of genotype information from these candidate gene SNPs into genotyping arrays for improvement of genomic estimates of DPR.

Conclusion

A large proportion of the SNPs previously related to DPR are predictive for DPR in a separate population. Use of these SNPs may increase the reliability of genomic estimates for DPR.

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