Variants in the pregnancy-associated plasma protein-A2 gene on *Bos taurus* autosome 16 are associated with daughter calving ease and productive life in Holstein cattle

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ABSTRACT

Reproductive disorders in dairy herds have a negative effect on farm profitability and sustainability of milk production. Given the substantial evidence of the role of the pregnancy-associated plasma protein (PAPP) gene family in the regulation of reproduction in humans and mice, its role in insulin-like growth factor metabolism, quantitative trait loci effects in the mouse, and location of a calving ease QTL on bovine chromosome 16, the *PAPP-A2* gene was chosen as a candidate gene to perform an association study for reproductive health in cattle. Single nucleotide polymorphisms (SNP) were identified in coding and conserved noncoding regions of the *PAPP-A2* gene in 3 dairy breeds. A total of 7 tag SNP were genotyped in 662 Holstein bulls (UCD-bulls) to perform marker trait association analysis. Three SNP (SNP 13, 15, and 16) were in strong linkage disequilibrium in Holsteins, showing significant positive associations with daughter calving ease, productive life, milk yield, and protein yield. These results were validated by genotyping SNP15 in a larger population of 992 bulls from the cooperative dairy DNA repository (CDDR-bulls). Our results demonstrate that the *PAPP-A2* gene is associated with reproductive health in Holstein cattle and that the identified SNP can be used as genetic markers in dairy breeding due to their positive association with reproductive and productive traits. Functional studies need to be conducted to identify the mechanisms for the association of SNP with these traits.

Key words: *PAPP-A2*, reproduction quantitative trait loci, association study

INTRODUCTION

Reproductive health in dairy cattle has decreased dramatically in recent years, paralleled by significant

increases in performance of production traits (López-Gatius, 2003). Negative correlations reported between performance and reproductive traits are a clear indication of this antagonistic relationship (Veerkamp and Beerda, 2007). The proportions of stillborn calves in both primiparous and multiparous Holstein cows have increased (Meyer et al., 2001), resulting in significant economic loss to the industry (Beerda et al., 2008, Heins et al., 2006). Calving difficulty (dystocia) was found to be a major determinant for the increase in incidence of stillbirths (Meyer et al., 2001) and also has a negative effect on milk yield and productive life (López de Maturana et al., 2007, Dematawewa and Berger, 1997). Thus, calving difficulty, measured as calving ease score, is considered one of the most economically important functional traits in dairy cattle (López de Maturana et al., 2007) and together with other reproductive traits, it has been incorporated in the population selection indices (VanRaden, 2004). However, the low heritability and the difficulty in defining a specific phenotype have hampered the genetic improvement of these traits (Kühn et al., 2003). Therefore, it is important that the underlying genetic determinants regulating reproductive traits in dairy cattle are identified.

Pappalysin-2, encoded by the pregnancy-associated plasma protein A2 (*PAPP-A2*) gene, is a metalloproteinase that specifically cleaves IGF-binding protein 5 (*IGFBP-5*) and increases the availability of IGF-I and IGF-II (Yan et al., 2010). This gene has been experimentally associated with growth and reproductive traits. Christians et al. (2006) fine-mapped a QTL affecting the skeletal growth in mice on murine chromosome 1, and the *PAPP-A2* gene was identified as the candidate gene for this QTL effect. Mouse gene knockout studies performed on the *PAPP-A* gene, the homolog of *PAPP-A2*, revealed that *PAPP-A* knockout mice are 60% the size of the wild-type control at birth (Conover et al., 2004) and have a demonstrated reduction in ovarian function and fertility (Nyegaard et al., 2010). Several recent studies in humans indicate an increase in the expression of the *PAPP-A2* gene and an increase in the level of pappalysin-2 in the serum of patients with

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gestational hypertension, which is a main cause for stillbirths (Wang et al., 2009; Winn et al., 2009). Studies conducted on the *PAPP-A* gene have also shown low levels of *PAPP-A* gene expression in humans with reproductive anomalies (Smith et al., 2002). In Holstein cattle, a QTL for calving ease has been detected on chromosome 16 (BTA16) in a region encompassing the location of the *PAPP-A2* gene (Schnabel et al., 2005).

Considering the role of PAPP genes in the regulation of reproduction in humans, QTL effects in mice, and location of calving ease QTL in cattle, *PAPP-A2* was hypothesized as a strong candidate gene for reproductive health in dairy cattle. In the present study, the coding and conserved noncoding regions of the *PAPP-A2* gene were resequenced in different dairy cattle breeds, SNP were identified and marker trait associations were examined in 2 Holstein cattle populations.

MATERIALS AND METHODS

Resequencing and SNP Detection

The genomic sequence for the bovine *PAPP-A2* gene (GenBank accession number: NC_007314.3, Ensembl ID: ENSBTAG00000018303) was obtained from the bovine Btau3.1 assembly (<http://www.ncbi.nlm.nih.gov/genome/guide/cow/index.html>). Conserved non-coding regions of the *PAPP-A2* gene were identified using Genome VISTA (Couronne et al., 2003) with genomic alignments to human, mouse, rat, dog, opossum, fugu, and chicken. Coding regions, conserved noncoding regions, 1,000 bp upstream from the transcription start site and 500 bp downstream from the stop codon (total of 8,550 bp) were resequenced (SeqWright DNA Technology Services, Houston, TX). Resequencing was performed in a dairy DNA resource panel specifically developed for SNP discovery (Rincon et al., 2007). In brief, this panel consists of 8 animals of each of the 3 major dairy breeds (Holstein, Jersey, and Brown Swiss) that are unrelated at least 3 generations back in their pedigrees.

Polymorphisms among and within breeds were analyzed using CodonCode aligner software (<http://www.codoncode.com>) and gene sequences and SNP were assembled and annotated in Vector NTI advance 10.1.1 software (Invitrogen, Carlsbad, CA). Linkage disequilibrium (LD) regions were identified using Haploview software (Barrett et al., 2005) with a minimum nonmissing genotype percentage of 80 for each marker, Hardy Weinberg *P*-value cutoff of 0.01, and minimum minor allele frequency of 0.01. Tag SNP were determined with the Tagger tool of Haploview with a correlation coefficient (r^2) greater than 0.8 and a log of odds threshold of 3.

Animal Populations and Genotyping

Two bull populations were used in the study. In order to minimize the population stratification and batch effects, the threshold for maximum number of bulls with the same sire was set to 10 in both populations and animals were selected according to this criterion. The first population consisted of 672 bulls belonging to the University of California Davis archival collection of Holstein bull DNA samples (UCD-bulls; Rincón et al., 2009b), and consists of bulls born between 1981 and 2003. More than 90% of the 672 bulls were born before the year 2000. The second population consisted of 992 bulls belonging to the cooperative dairy DNA repository (CDDR), which is maintained by the USDA Bovine Functional Genomics Laboratory (Beltsville, MD). The CDDR population (CDDR-bulls) consisted of bulls born between 1988 and 2006, and more than 75% of the bulls were born after the year 2000. A total of 7 tag SNP (Table 1) were genotyped in the UCD-bulls population ($n = 672$) using the Sequenom MassARRAY(R) platform (GeneSeek Inc., Lincoln, NE). In order to validate the results from the association analysis obtained from the UCD-bulls population, one significant SNP (SNP 15; Table 1) was genotyped in the CDDR-bulls population using the KASPar (Kbiosciences, Hertsfordshire, UK) genotyping system.

Association Analysis

Seven management traits: productive life (PL), SCS, daughter pregnancy rate (DPR), sire calving ease, daughter calving ease (DCE), sire stillbirth, daughter stillbirth, and 5 productive traits: milk yield (MY), fat yield; protein yield (PY), fat percentage, and protein percentage were considered for the association analysis. For each bull, PTA and reliability values for these traits were obtained from the animal improvement program laboratory (AIPL; <http://www.aipl.arsusda.gov/>) January 2010 release of bull evaluations. In this evaluation, PTA values are expressed as deviations from the Holstein base of animals born in the year 2005 (AIPL, Beltsville MD; <http://aipl.arsusda.gov/reference/base2010.htm>). To increase the accuracy in the association study, only bulls with reliability values for individual traits greater or equal to 60% were included in the analysis. Very high positive correlations (above 0.85) were observed between the MY and the PY in both UCD-bulls and CDDR-bulls populations. In both populations, the PL had positive correlations ranging from 0.09 to 0.19 with the MY and the PY. High positive correlations (0.59 and 0.60) were observed between the PL and the DPR in both populations, whereas high negative correlations (−0.36 and −0.41) were observed

Table 1. Fifteen SNP in the *PAPP-A2* gene identified in Holstein animals by resequencing

NCBI ID ¹	Code ID ²	Location on BTA16 (bp) ³	Alleles ⁴	SNP description
ss115492409	SNP1	55,274,969	A/G	Noncoding
ss115492410	SNP2	55,275,066	A/G	Noncoding
ss115492411	SNP3	55,275,112	A/G	Noncoding
ss115492416	SNP5	55,275,732	C/T	Noncoding
ss115492423	SNP6	55,276,698	C/T	Synonymous
ss115492424	SNP7	55,276,776	A/G	Synonymous
ss115492429	(SNP8)	55,277,286	C/T	Synonymous
ss115492438	(SNP10)	55,508,858	A/C	Ala/Glu
ss115492448	(SNP13)	55,517,243	A/T	Noncoding
ss115492449	(SNP14)	55,517,276	A/C	Noncoding
ss115492450	(SNP15)	55,519,211	G/T	Noncoding
ss115492451	(SNP16)	55,519,429	A/G	Gly/Asp
rs42301985	(SNP17)	55,548,447	A/G	Gln/Arg
rs42302737	SNP18	55,578,003	C/T	Pro/Ser
ss115492454	SNP19	55,583,002	C/T	Noncoding

¹ss = dbSNP assigned submitter SNP ID; rs = dbSNP assigned reference SNP ID.

²ID assigned to the SNP in order of their location in the *PAPP-A2* gene. Tag SNP used for genotyping and association analysis are in parentheses.

³SNP locations according to *Bos taurus* assembly Btau 4.0.

⁴A = adenine; T = thymine; C = cytosine; G = guanine.

between the PL and the DCE. Three reproductive traits (DPR, DCE, and daughter stillbirth) showed negative correlations ranging from -0.12 to -0.33 with the productive traits (MY and PY) in both populations.

Marker-trait association analysis was performed using a linear regression test under an additive model assumption. Statistical analyses were performed using the genotype association and regression modules from SNP variation suite (SVS) version 7 (Golden Helix Inc., Bozeman, MT). The regression module corresponds to the additive allele substitution model. For a purely additive genetic situation, this model provides higher power than does the general linear model (GLM) used in the literature for similar analyses (Cobanoglu et al., 2006; Khatib et al., 2006). A similar approach was successfully used to analyze tag SNP on BTA4 associated with milk production traits (Rincón et al., 2009b) and to analyze the association of tag SNP within the bovine *STAT6* gene and carcass traits in feedlot cattle (Rincon et al., 2009a). Then principal component analysis (PCA) was used to correct for stratification and batch effects (Patterson et al., 2006; Price et al., 2006). A total of 222 SNP were used in PCA in the UCD-bulls population. These SNP consist of the 7 tag SNP in the *PAPP-A2* gene and 215 other SNP randomly distributed across the bovine genome. These SNP were identified in several earlier resequencing projects in our laboratory (Rincon et al. 2007, 2009a,b,c; J. F. Medrano, unpublished data) and along with *PAPP-A2* SNP they were genotyped in the UCD-bulls population. False discovery rate (FDR) was calculated using the SVS algorithm (Storey, 2002). The cutoff for significant association was set at FDR q value < 0.2 .

RESULTS AND DISCUSSION

The study design for the characterization and association analysis of the *PAPP-A2* gene consisted of 3 steps; SNP discovery, association study and validation. In the first step, the coding and noncoding conserved regions of the bovine *PAPP-A2* gene were sequenced in a dairy breed panel (Holstein, Jersey, and Brown Swiss) and 19 SNP were identified. Holstein animals contained 15 of these SNP (Table 1) with 8 SNP located in noncoding regions and 7 located in the coding region. Four of the coding SNP were nonsynonymous. Thirteen of the polymorphisms in Holstein animals were novel and they were submitted to dbSNP (www.ncbi.nlm.nih.gov/projects/SNP). Haploview analysis indicated 14 tag SNP in the dairy breed panel. Holsteins were polymorphic for only 7 tag SNP that were genotyped in the UCD-bulls population.

Regression analysis indicated a significant association ($P \leq 0.05$) for SNP 13, 15, and 16 (Table 1) with traits DCE, DPR, PL, MY, and PY (Table 2). These 3 SNP were in Hardy Weinberg equilibrium with a call rate above 98% and minor allele frequency above 0.2. They showed a strong LD in the Holstein population (SNP13 and SNP15 $r^2 = 0.95$; SNP13 and SNP16 $r^2 = 0.99$; SNP15 and SNP16 $r^2 = 0.95$). Bulls that are homozygous for the wild-type allele of these SNP (TT in SNP13, TT in SNP15, GG in SNP16) showed increases in mean phenotypic values for DPR, PL, MY, PY, and a decrease in mean DCE (Table 2).

When 2 SNP were in strong LD, genotyping only one of them was sufficient to obtain information in an association test (Xu et al., 2007; Haiman and Stram,

Table 2. Significant SNP associated with management traits, milk yield, and protein yield in the UCD-bulls and CDDR-bulls populations with PTA reliability $\geq 60\%$ for individual traits (significant level Hardy Weinberg equilibrium $P \leq 0.01$)

Population ¹	Marker	SNP MAF ²	Call rate	Trait ³	N ⁴	P-value ⁵	ASE ⁶	Genotype with mean and standard error		
UCD-bulls	SNP13	0.22	0.99	DCE	541	0.00490	0.7	TT 7.8 $\pm$ 0.08	AT 8.3 $\pm$ 0.10	AA 8.3 $\pm$ 0.4
				DPR	587	0.00004	-0.8	AA -0.8 $\pm$ 0.20	AT -0.7 $\pm$ 0.09	TT -0.3 $\pm$ 0.7
				PL	672	0.00280	-0.9	AA -0.9 $\pm$ 0.30	AT 0.1 $\pm$ 0.10	TT 0.2 $\pm$ 0.1
				MY	672	0.01170	-99.3	AA 128 $\pm$ 48	AT 177 $\pm$ 19	TT 227 $\pm$ 14
				PY	672	0.00386	-3.0	AA 6.3 $\pm$ 1.4	AT 6.8 $\pm$ 0.45	TT 8.4 $\pm$ 0.4
	SNP15	0.23	0.98	DCE	541	0.00205	0.7	TT 7.8 $\pm$ 0.08	GT 8.3 $\pm$ 0.10	GG 8.5 $\pm$ 0.4
				DPR	587	0.00015	-0.7	GG -0.9 $\pm$ 0.20	GT -0.7 $\pm$ 0.09	TT -0.3 $\pm$ 0.07
				PL	672	0.00234	-0.8	GG -1.1 $\pm$ 0.3	GT -0.0 $\pm$ 0.10	TT 0.2 $\pm$ 0.1
				MY	672	0.00530	-102	GG 104 $\pm$ 47	GT 188 $\pm$ 19	TT 228 $\pm$ 14
				PY	672	0.00209	-3.1	GG 5.4 $\pm$ 1.4	GT 6.8 $\pm$ 0.45	TT 8.5 $\pm$ 0.4
	SNP16	0.23	1.00	DCE	541	0.00413	0.7	GG 7.8 $\pm$ 0.08	AG 8.3 $\pm$ 0.10	AA 8.4 $\pm$ 0.4
				DPR	587	0.00009	-0.7	AA -0.8 $\pm$ 0.2	AG -0.7 $\pm$ 0.01	GG -0.3 $\pm$ 0.07
				PL	672	0.00158	-0.8	AA -0.9 $\pm$ 0.2	AG -0.1 $\pm$ 0.1	GG 0.2 $\pm$ 0.1
				MY	672	0.01147	-93.9	AA 136 $\pm$ 47	AG 180 $\pm$ 19	GG 228 $\pm$ 14
				PY	672	0.00416	-3.0	AA 6.3 $\pm$ 1.4	AG 6.8 $\pm$ 0.45	GG 8.4 $\pm$ 0.4
CDDR-bulls	SNP 15	0.18	1.00	DCE	848	0.01465	0.4	TT 7.5 $\pm$ 0.06	GT 7.8 $\pm$ 0.2	GG 8.2 $\pm$ 0.1
				PL	976	0.00013	-0.9	GG -0.7 $\pm$ 0.3	GT -0.1 $\pm$ 0.1	TT 0.3 $\pm$ 0.09
				MY	992	0.01784	-87.5	GG 81 $\pm$ 51.7	GT 218 $\pm$ 21	TT 237 $\pm$ 12
				PY	992	0.00159	-0.3	GG 3.2 $\pm$ 1.4	GT 7.3 $\pm$ 0.45	TT 8.2 $\pm$ 0.32

¹UCD-bulls = bulls belonging to the University of California Davis archival collection of Holstein bull DNA samples; CDDR-bulls = bulls belonging to the cooperative dairy DNA repository.

²MAF = minor allele frequency.

³DCE = daughter calving ease measured as percentage difficult births in primiparous heifers (% DBH); DPR = daughter pregnancy rate (%); PL = productive life (mo); MY = milk yield (kg); PY = protein yield (kg).

⁴Number of animals with PTA reliability $\geq 60\%$.

⁵Regression P -value ($P \leq 0.05$, false discovery rate ≤ 0.20).

⁶Allele substitution effect.

2008). Thus, in order to validate the results of the association analysis in the UCD-bulls population, SNP15, which is in strong LD with SNP 13 and 16, was selected to genotype the larger CDDR-bulls population. In this population, SNP 15 was in Hardy Weinberg equilibrium with a call rate of 1.0 and minor allele frequency of 0.18. A significant association with the same specific genotypes and phenotypes was observed for DCE, PL, MY, and PY (Table 2). These results validated the observations in the UCD-bulls population and confirmed the association of DCE, PL, MY, and PY with SNP15. Daughter pregnancy rate did not show a significant association in the CDDR-bulls population, which consisted mainly of recently proven progeny tested bulls with fewer daughters. Because of the low heritability ($h^2 = 0.04$) of reproductive traits, a large group of daughters is necessary to obtain high reliability values for traits like DPR (Dechow et al., 2004). Compared with the UCD-bulls population, mean DPR reliability was relatively low in the CDDR-bulls population (UCD-bulls mean DPR reliability = 0.82, CDDR-bulls mean DPR reliability = 0.70). In UCD-bulls, 46% of the population had DPR reliability above 0.9, whereas in the CDDR-bulls population only 19% of the bulls had DPR reliability above 0.9. The low heritability of DPR jointly with the low reliability of DPR proofs is

likely responsible for the nonsignificant association of SNP15 in the CDDR-bull population.

The *PAPP-A2* gene has 443,513 bp with 22 exons located in bovine chromosome 16 (Figure 1). A Holstein QTL for calving ease spans the region between 55.3 and 72.2 cM, centered at 56.2 cM in BTA16 (Schnabel et al., 2005). The *PAPP-A2* gene is located within this QTL region (between 55.2 and 55.6 cM). Genes *ASTN*, *FAM5-B*, *GLRX*, *LZTR2*, and *RASAL2* are located in the same QTL region on BTA16. Evaluation of these genes indicated that *ASTN* and *FAM-5B* encode for neuronal tissue-specific proteins, *GLRX* encodes for an enzyme with glutathione-disulfide oxidoreductase activity, *LZTR2* encodes a protein required for endoplasmic reticulum secretory mechanisms, and *RASL2* encodes for an inhibitory regulator of the Ras-cyclic AMP pathway (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>). None of these genes are associated with body size phenotypes in the mouse genome informatics (MGI) database (<http://www.informatics.jax.org/>). However, *PAPP-A2* is identified as a candidate gene for skeletal growth in mice and considering the important function that pappalysin-2 plays in regulating the availability of IGF, *PAPP-A2* stands out as a logical candidate gene for the calving ease QTL. This is supported by the strong association of SNP 13, 15, and

Figure 1. Diagram showing the genomic location of the *PAPP-A2* gene, genomic structure, and identified polymorphisms: (A) 2-Mb genomic region on bovine chromosome 16 (assembly Btau 4.0), *PAPP-A2* (black arrow), other genes in close vicinity (gray arrows). Arrow directions indicate the coding orientation of the genes; (B) bovine *PAPP-A2* gene structure comprising 22 exons (E1–E22). The locations of SNP described in Table 1 are indicated with downward dashed arrows.

16 with DCE PTA in this study. Additionally, in Holstein populations, QTL with large confidence intervals are reported in BTA16 for PL (centered at 77.5 cM; Ashwell et al., 1998), PY, and MY (centered at 46 cM; Daetwyler et al., 2008).

As stated above, a strong association was found between the polymorphisms of the *PAPP-A2* gene and DCE. Even though the biological role of *PAPP-A2* in determining DCE is still unknown, it can be hypothesized that *PAPP-A2* might be regulating the maternal pelvic size, which is a major determinant of daughter calving difficulty (Mee, 2008). A QTL affecting skeletal growth and body size in mice was fine-mapped to murine chromosome 1, and the *PAPPA-2* gene was identified as the candidate gene for this QTL effect (Christians et al., 2006). Gene knockout mice of the *PAPP-A* gene, the homolog of *PAPP-A2*, are at birth 60% the size of wild-type controls and they showed a significant delay in fracture healing. These mouse studies highlight the importance of *PAPP* genes as modulators of skeletal growth through their IGFBP protease activity regulating IGF action (Conover et al., 2004; Nyegaard et al., 2010). In humans, *PAPP-A2* has been associated with gestational hypertension, which is a main cause for still-birth and dystocia. Several recent studies have shown an increase in the expression of the *PAPP-A2* gene and increases in the level of pappalysin-2 in the serum of patients with gestational hypertension (Nishizawa et al., 2008; Wang et al., 2009; Winn et al., 2009). No published research on hypertension and dystocia in cattle exists, and the exact role that *PAPP-A2* plays

in the determination of calving ease in cattle awaits further investigation.

Another important discovery of these 3 polymorphic markers (SNP 13, 15, and 16) is that the same allele showed a positive association with MY and PY. Negative genetic correlations reported between reproduction and production traits have made the breeder cautious in selecting markers for marker-assisted selection (MAS; Simianer et al., 1991; Castillo-Juarez et al., 2000). The *STAT5A* gene has the first identified SNP with positively correlated associations between production and reproduction traits. In *STAT5A* a polymorphism in an exon is associated with low embryonic survival and decrease in milk protein and fat percentages (Khatib et al., 2008). A similar association pattern was found in the present study and the 3 SNP in *PAPP-A2* are of particular value in MAS due to their favorable associations with calving ability, milk yield, and protein yield. No published research on the significance of the *PAPP-A2* gene in lactogenesis exists. However, it is known that IGFBP-5 produced from the mammary gland during the involution stage promotes apoptosis by capturing and sequestering IGF that enhance mammary epithelial cell proliferation and survival (Moorehead et al., 2001; Allan et al., 2004). As *PAPP-A2* is responsible for proteolysis of IGFBP-5, it may be playing a role in inhibiting the action of the binding protein during development and proliferation of mammary epithelial cells, thus increasing the availability of IGF-I and II, which is essential for the mitosis of mammary epithelial cells. This could explain the association of the *PAPP-A2* gene with milk

yield. It has been identified that IGF-1 stimulates the protein synthesis in bovine mammary epithelial cells by activating the mammalian target of rapamycin (mTOR) signaling pathway (Burgos and Cant, 2010). Thus, the increase in availability of IGF-1 by *PAPP-A2* can enhance protein synthesis in the mammary gland. However the exact role of *PAPP-A2* in mammary gland development has not been documented in the literature and it is a promising area for research.

The role of the 3 SNP affecting the functionality of the *PAPP-A2* gene has not yet been investigated. Among the 3 polymorphisms, SNP 16 is nonsynonymous with glycine to aspartic acid substitution (Gly1254Asp). The other 2 polymorphisms are in intron 10 (Figure 1), with SNP 13 located 15bp away from a splice donor site and SNP 15 located 104bp away from a splice acceptor region. It is possible that one of the 3 polymorphisms has a regulatory effect on the expression of the *PAPP-A2* gene or that the 3 SNP are in LD with a *cis* or *trans* regulatory region of the *PAPP-A2* gene.

Daughter calving ease, which is a measure of the intensity of dystocia, is one of the most economically important traits for reproductive health in dairy cattle (Dematawewa and Berger, 1997). High incidence of dystocia reduces profitability, reproductive performance, and milk production. Problems with calving ease increase the culling rate of the herd and substantially decrease longevity (López de Maturana et al., 2007). Because of this, PL is strongly correlated with DCE, and DCE is included in the prediction of PL (AIPL; <http://www.aipl.arsusda.gov/reference/multi-pl.htm>). Thus, the association of these 3 SNP with DCE and PL suggests an important role of *PAPP-A2* for fertility genetic improvement in Holstein cows.

CONCLUSIONS

In this study, the *PAPP-A2* gene was selected as an important candidate gene for reproductive health in cattle by considering the functional role of PAPP genes in humans and QTL effects in bovine and mouse experimental results. The bovine *PAPP-A2* gene was characterized, and 3 SNP associated with DCE and PL were identified and validated in 2 Holstein bull populations. These polymorphisms showed positive associations with MY and PY which make them valuable genetic markers in dairy breeding. Functional studies, such as quantification of the expression of *PAPP-A2* in placenta and ovaries of animals with the different genotypes and proteomic analysis of pappalysin-2, need to be conducted to explore the mechanisms of these associations. The findings of this study provide new insights into the roles of *PAPP-A2* on reproduction and production traits in dairy cattle.

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