Pattern of Expression of the Uterine Milk Protein Gene and its Association with Productive Life in Dairy Cattle

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ABSTRACT

The uterine milk proteins (UTMP) are the major proteins secreted by the endometrium, primarily under the control of progesterone. Specific functions of UTMP are poorly understood, but may include protease inhibition, nutrition of the conceptus, growth control, and suppression of the maternal immune system. The uterine milk protein gene (*UTMP*) was chosen for this study because of its possible roles in health traits and results of previous studies on the association of the *UTMP* region with milk production and productive life in dairy cattle. Expression of *UTMP* was examined in 198 bovine tissues obtained from 10 fetuses and 17 cows. Transcripts of *UTMP* were found in all cotyledon tissues examined and to a lesser extent in ovary, pituitary, and spleen tissues obtained from fetuses. The *UTMP* gene was predominantly expressed in endometrium (17/17), ovary (15/16), and caruncle (12/12) tissues obtained from cows. The predominant expression of *UTMP* in reproductive tissues is consistent with an important role of this gene in reproductive success. To investigate the association between *UTMP* and production traits in cattle, we identified 2 synonymous single nucleotide polymorphisms (SNP) at positions 1179 (A/G) and 1296 (A/G) using the pooled DNA sequencing approach. The DNA was extracted from 28 Holstein sires and their 1,362 sons obtained from the Cooperative Dairy DNA Repository and from 913 cows from the University of Wisconsin resource population. Single nucleotide polymorphism 1296 was associated with a significant increase in productive life in both populations. This finding is similar to results previously obtained for the protease inhibitor gene, which is near *UTMP* and was also found to be associated with productive life in dairy cattle. Differential allelic expression of *UTMP* was observed in reproductive tissues obtained from 9 heterozygous individuals. The differential allelic expression observed in this study is consistent with other studies

showing a correlation between allelic variation in gene expression and phenotypic variability.

Key words: uterine milk protein gene, differential allelic expression, candidate gene, productive life

INTRODUCTION

The uterine milk proteins (**UTMP**) are the major proteins secreted by the endometrium, primarily under the control of progesterone (Moffatt et al., 1987). Specific functions of UTMP are poorly understood, but may include protease inhibition, nutrition of the conceptus, growth control, and suppression of the maternal immune system (Ing and Roberts, 1989). Stewart et al. (2000) showed that ovine intracaruncular endometrial *UTMP* mRNA levels increased about 3-fold between d 20 and 60 of gestation, increased another 3-fold between d 60 and 80, and then declined slightly to d 120. When using Western blotting and immunohistochemistry, goat UTMP has been localized to the glandular epithelium of the endometrium at d 25 of pregnancy (Tekin et al., 2005). Leslie et al. (1990) found that UTMP was detected in the uterine fluid and secreted by the endometrium of pregnant cows. However, at present, it is not known whether the bovine *UTMP* gene is expressed in bovine tissues other than the endometrium. Our study aimed to investigate the distribution of *UTMP* transcripts in a wide range of fetal and adult bovine tissues.

The *UTMP* gene was chosen for this study because previous studies of the *UTMP* region have shown an association with productive life (**PL**) and milk composition traits in dairy cattle. Heyen et al. (1999) reported an association between marker ILSTS054 and PL in the North American Holstein population. Khatib et al. (2005) reported a significant association between the protease inhibitor gene (**PI**) and PL and milk composition traits in Holstein dairy cattle. In addition, both *PI* and *UTMP* have protease-inhibition functions, and the genes are both located on chromosome 21, within a 321.6-kb genomic region. Thus, possible roles of *UTMP* in health traits and its proximity to *PI* were the motives for investigation of possible effects of *UTMP* on health traits in dairy cattle.

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Recent studies in humans (Yan et al., 2002; Bray et al., 2003; Lo et al., 2003; Pastinen et al., 2003), in mice (Cowles et al., 2002), and in corn (Guo et al., 2004) have shown that alleles of nonimprinted genes are not expressed equally at the mRNA level, and such differential allelic expression (**DAE**) may be the basis for variation in disease susceptibility and in determining phenotypic diversity. Yan et al. (2002) examined single nucleotide polymorphisms (**SNP**) for 13 genes in 96 individuals and found significant differences in allelic variation in 6 out of the 13 genes studied, with 1.3- to 4.3-fold differences between alleles. In a survey of allelic variation among human genes, Pastinen et al. (2003) selected 129 genes based on their potential function in the immune system and metabolic disorders and identified 18% of the genes with deviations from the equimolar ratio between the 2 alleles. Lo et al. (2003) examined allele-specific gene expression of 1,063 transcribed SNP by using the Affymetrix HuSNP chip system. The authors found that among the 602 genes that were heterozygous, 326 showed preferential expression of 1 allele in at least 1 individual, and 170 of those genes showed a greater than 4-fold difference between the 2 alleles.

Accumulating evidence indicates that DAE is associated with phenotypic variability of quantitative and qualitative traits. For example, Hirota and colleagues (2004) studied the relationship between DAE of the human cytochrome P450 3A4 (**CYP3A4**) gene and metabolic activity in the liver. Individuals with a large difference in expression levels between the 2 alleles showed reduced total **CYP3A4** mRNA and, hence, the total metabolic activity of the gene was reduced. That was the first study to demonstrate that **CYP3A4** expression levels were affected by allele-specific expression levels.

In this study, we analyzed the expression pattern of **UTMP** in fetal and adult cattle tissues and investigated its association with production traits in 2 independent Holstein populations. In addition, we examined the relationship between DAE of **UTMP** and association with quantitative traits.

MATERIALS AND METHODS

Tissue Collection and Nucleic Acid Extraction

Tissues from 10 fetuses and 17 pregnant cows were obtained from a local slaughterhouse. For 9 of 10 fetuses, their dams were included in this study. Fetal tissues included lung, brain, bone, eye, heart, skeletal muscle, liver, cotyledon, spleen, pituitary, kidney, intestine, pancreas, mammary gland, testis, ovary, hypothalamus, and cartilage. The fetuses were from 55 to 100 d postconception. The tissues from cows included endometrium, ovary, oocytes, liver, heart, spleen, kid-

ney, caruncle, pancreas, lung, and skeletal muscle. After dissection, tissues were immediately chilled on ice and submerged in RNeasy RNA stabilizing reagent (Qiagen, Valencia, CA). Total RNA was extracted using the RNeasy kit (Qiagen) and treated with DNase I (Sigma, St Louis, MO). Then, DNA was extracted by grinding 30 to 100 mg of one tissue from each individual using the AquaPure Genomic DNA kit (Bio-Rad, Hercules, CA).

Reverse Transcription PCR

Reverse transcription (**RT**) of the bovine **UTMP** gene was performed using the primers UMP1 (ATGTCCCA CGGAGAATGAA) and UMP2 (CCTCCTTCTGGGTG AGTTTG). The temperature cycles were as follows: 50°C for 30 min (reverse transcription); 95°C for 15 min, followed by 33 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min; and a final extension at 72°C for 10 min. Primers β -actinF (CAGCACAATGAAGAT CAAGATCATC) and β -actinR (AAAGGGTGTAACGC AGCTAACAGT) were used to amplify 191 bp from the housekeeping gene β -actin (GenBank accession number NM_173979) cDNA. The RT-PCR products were electrophoresed on a 1.5% agarose gel. The detectable amplification of any product was interpreted as a positive indication of **UTMP** expression; no formal quantitative test was performed. Primers β -actinF and β -actinR were used to amplify the housekeeping gene β -actin as a control for the expression of **UTMP** in the various tissues.

Phenotypic Data

The association between **UTMP** variants and production traits was studied in 2 independent Holstein populations: the Cooperative Dairy DNA Repository (**CDDR**) population of AI sires and cows from the University of Wisconsin resource population (henceforth, UW resource population). The UW population was created to search (using a daughter design) 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 having 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. Phenotypic data for both analyses were obtained from the Animal Improvement Programs Laboratory (Beltsville, MD). The CDDR data were PTA for milk yield, milk protein and fat yields, milk protein and fat percentages, PL in months, and

SCS from August 2005. The phenotypic data from the UW resource population comprised yield deviations for milk yield, milk protein and fat yields, and PL. Productive life is a longevity trait defined as a cow's total lifetime months in milk with limits of 10 mo per lactation and 7 yr of age (VanRaden and Wiggans, 1995). Deviated records of PL were based on direct observations and also correlated traits measured earlier in life. The average reliability of PTA for PL in the CDDR population was 71%.

Polymorphism Detection and Genotyping

Semen samples from 28 Holstein sires and their 1,362 sons (23 to 104 sons per sire) were obtained from the CDDR, which is maintained by the USDA Bovine Functional Genomics Laboratory. In addition, blood samples were obtained from 913 cows from the UW resource population. For a detailed description of this population, see Cobanoglu et al. (2006). Genomic DNA was extracted from semen and blood samples using standard procedures. To detect SNP in the *UTMP* gene (GenBank accession number L22095), DNA pools were constructed from 220 bovine samples and amplified with the primers UTMP3 (GGCCCTACATCAAGCTGAGA) and UTMP4 (CTACTCAACTTGGGGGTTGA) as previously described (Leonard et al., 2005). The PCR products of the pooled DNA samples were sequenced and SNP were identified by visually inspecting sequence traces. For individual genotyping, primers UTMP3 and UTMP4 were used to amplify a 327-bp fragment of *UTMP* from genomic DNA. The PCR products were subjected to RFLP detection using the restriction enzyme *Bsr*I that distinguishes alleles A and G of the SNP at position 1296. The digestion products were electrophoresed on a 3.0% agarose gel.

Allele Expression Quantification

Differential allelic expression of the *UTMP* gene was quantified by a sequencing-based approach and by single-nucleotide primer extension analysis. In the sequencing approach, RT-PCR products amplified from heterozygous individuals were sequenced according to standard procedures. Sequencing reactions of PCR amplicons were performed for 50 cycles at 96°C for 10 s, 58°C for 4 min, and a final extension at 72°C for 7 min. Data were analyzed using Applied Biosystems' Sequencing Analysis (version 5.0; Applied Biosystems, Foster City, CA). The SNP were identified by visually inspecting each base in sequencing traces. Allelic variation was estimated by measuring the proportions of the peak heights of the 2 alternative alleles of the SNP. In the primer extension approach (Norton et al., 2002),

RT-PCR products were purified from agarose gel using the GFX PCR DNA purification Kit (Amersham Biosciences, Piscataway, NJ). Primer extension reactions were prepared in a total volume of 10 μ L containing 1 μ L of purified RT-PCR product, 5 μ L of SnapShot Kit (Applied Biosystems), 0.02 μ M of extension primer, and 1 μ L of deionized water. The primer extension reactions were subjected to 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. In a postextension treatment, reactions were treated with 1 unit of shrimp alkaline phosphatase at 37°C for 1 h followed by deactivation of the enzyme at 75°C for 15 min. The primer 1179.ext (GTGAAGGCAAAGGAGGTCCC) was used in the primer extension reactions. Samples were electrophoresed on a 3700 DNA sequencer (PE Applied Biosystems) and data were analyzed by using Genescan Analyzer version 2.5 software (PE Applied Biosystems). Allelic ratios were estimated by measuring the proportions of the peak heights of the 2 fluorescently labeled variants of the SNP found at the same position. No significance tests were performed.

Statistical Analysis

For the CDDR population, data relative to each trait were analyzed using an allele substitution model expressed as

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

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 G alleles (0, 1, or 2) on 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.

Data relative to the daughters in the UW resource population were analyzed using 2 models. First, an allele substitution model similar to the CDDR described above was considered, which is expressed as

$$y_{ijk} = \mu + s_i + p_{ij}\tau + \beta x_k + \varepsilon_{ijk},$$

where y_{ijk} represents the yield deviation of daughter j of sire i , τ is an effect associated with *Mycobacterium paratuberculosis* infection status, and p_{ij} is an indicator variable assuming the values 0 and 1 for noninfected and infected cows, respectively. The index k indicates the genotype of the cows ($k = AA, AG, \text{ or } GG$), and x_k is the number of G alleles in the genotype k ($x_k = 0, 1, \text{ or } 2$, respectively). The remaining terms in the model are as previously defined. *Mycobacterium paratubercu-*

Table 1. Expression pattern of uterine milk protein gene (*UTMP*) transcripts obtained from various fetal tissues¹

Fetus (age in d)	Tissues expressing <i>UTMP</i>	Tissues not expressing <i>UTMP</i>
1 (55)		Eye, heart, muscle, liver, lung, brain, bone
2 (65)	Cotyledon, pituitary	Spleen, eye, lung, heart, kidney, intestine, pancreas, muscle, liver, bone, brain, mammary gland
3 (68)	Cotyledon	Heart, muscle, eye, spleen, bone, lung, liver, kidney, testis, pituitary, pancreas, brain
4 (68)	Cotyledon	Mammary gland
5 (70)		Heart, muscle, brain, spleen, ovary
6 (70)		Liver, lung, brain, muscle, intestine, eye, bone, heart, kidney, ovary, pancreas, mammary gland
7 (75)		Lung, brain, eye, bone, testis, heart, kidney, muscle, pituitary, spleen, liver
8 (85)	Cotyledon	Pituitary, spleen, lung, heart, brain, kidney, liver, muscle, adrenal, mammary gland, ovary
9 (90)	Cotyledon	Kidney
10 (100)	Ovary, pituitary, hypothalamus, spleen	Lung, heart, pancreas, kidney, liver, bone, cartilage, mammary gland, muscle

¹Not all tissue types were examined in all fetuses.

losis infection status was included in the model because the UW population was originally created to search for genetic markers associated with susceptibility to paratuberculosis.

In addition, a second model was used for each trait, which is described as

$$Y_{ijk} = \mu + S_i + P_{ij} + g_k + \varepsilon_{ijk},$$

where g_k is the effect of the *UTMP* genotype k . Additive genetic effects were estimated as half the difference between the 2 homozygous groups, and dominance effects were estimated as the difference between the average of the 2 homozygous groups and the heterozygous group. The AA genotype was set as baseline for estimating the genotypic effects. All the analyses were implemented using the GLM procedure of SAS (SAS Institute, 1999).

RESULTS

Tissue Distribution of *UTMP* Expression in Fetuses and Cows

Table 1 shows the expression of *UTMP* in 93 fetal tissues. The *UTMP* gene was expressed in all 5 cotyledon tissues examined. Two pituitary tissues expressed *UTMP*; 1 tissue from fetus 2 (d 65) showed a low expression level. Ovary, hypothalamus, and spleen tissues showed low levels of expression in fetus 10 (d 100). Expression of *UTMP* was not detected in eye, heart, skeletal muscle, lung, bone, kidney, pancreas, testis, mammary gland, adrenal, or intestine tissues in any of the fetuses examined. Table 2 shows the expression status of *UTMP* in 107 tissues obtained from 17 cows; *UTMP* was predominantly expressed in endometrium (17/17), ovary (15/16), and caruncle (12/12) tissues. Inconsistent amplification of *UTMP* was shown in liver, oocytes, spleen, and lung tissues. The *UTMP* gene was

expressed in the livers of 3 cows out of 6 examined; 2 liver tissues showed low expression levels; *UTMP* was also expressed in 1 oocyte tissue out of 12 examined. Heart, skeletal muscle, kidney, and pancreas tissues did not express the *UTMP* gene in any examined individual.

Association of *UTMP* with Productive Life in Two Independent Holstein Populations

Using the pooled DNA sequencing approach, an A/G SNP at position 1179 and an A/G SNP at position 1296 were identified in bovine *UTMP*. Genotyping of random samples revealed low polymorphism at SNP 1179; therefore, it was not further analyzed in this study. For SNP 1296, the frequencies of allele G in the CDDR and UW populations were 0.72 and 0.64, respectively. The association between *UTMP* variants at position 1296 and production traits was tested in 1,363 bulls from the CDDR and in 913 cows from the UW resource Holstein dairy cattle populations. Single nucleotide polymorphism 1296 was associated with a significant increase in PL in both populations. For the CDDR population, allele G was associated with a significant increase in PL ($P = 0.008$). Estimated allele substitution effects and their standard errors for production and health traits are given in Table 3. A bull inheriting allele G vs. allele A is expected to have, on average, 0.26 mo greater PTA for PL. For the UW resource population, allele G had an average substitution effect of 1.44 mo of PL ($P = 0.033$). The *UTMP* variants did not show significant associations with milk, fat, or protein yields or SCS in either population studied (Table 3). Dominance and additive genetic effects were estimated for PL and other traits using the UW resource population data. Dominance effects were not statistically significant for any trait. An additive genetic effect was significant only for PL ($P = 0.049$), with an estimate of 1.34 ± 0.68 mo.

Table 2. Expression pattern of uterine milk protein gene (*UTMP*) transcripts obtained from various pregnant cows' tissues¹

Cow (estimated d into gestation)	Tissues expressing <i>UTMP</i>	Tissues not expressing <i>UTMP</i>
1 (100)	Endometrium, ovary	Heart, kidney
2 (100)	Endometrium, spleen, liver,	Heart, ovary, oocytes, kidney, lung
3 (85)	Endometrium, ovary, caruncle	Spleen, kidney
4 (70)	Endometrium, ovary	Oocytes, pancreas, lung
5 (55)	Endometrium, spleen	Liver, heart, muscle, oocytes, ovary, lung
6 (75)	Endometrium, ovary, caruncle, liver	Heart, kidney, oocytes, spleen
7 (68)	Endometrium, ovary, caruncle,	Muscle, liver, lung, oocytes, heart, spleen
8 (65)	Endometrium, ovary, caruncle, lung	Heart, muscle, liver, oocytes, pancreas
9 (90)	Endometrium, ovary	Oocytes
10 (68)	Endometrium, caruncle, ovary	Oocytes
11 (90)	Endometrium, ovary, caruncle, oocytes, lung, spleen	Pancreas
12 (85)	Endometrium, ovary, caruncle, spleen, liver	Heart, muscle, oocytes, pancreas, lung
13 (83)	Endometrium, caruncle, lung	
14 (90)	Endometrium, ovary, caruncle	Oocytes, lung, pancreas
15 (75)	Endometrium, ovary, caruncle,	Oocytes, lung, pancreas
16 (125)	Endometrium, caruncle, ovary, uterus	Heart, muscle, kidney
17 (80)	Endometrium, caruncle, ovary	Heart, muscle

¹Not all tissues types were examined in all cows.

Allelic Variation in *UTMP* Gene Expression

To detect heterozygous individuals for allelic variation analysis, primers UMP3 and UMP4 were used to amplify genomic DNA from 10 fetuses and 17 cows. Fetus 10 and cow 11 were heterozygous for the SNP (A/G) at position 1179, and 5 cows (cows 2, 8, 12, 16, and 17) were heterozygous for the SNP (A/G) at position 1296. Then, primers UMP1 and UMP2 were used to amplify RT-PCR products from endometrium, ovary, and caruncle tissues obtained from the informative individuals. Differential allelic expression was measured in tissues in which *UTMP* was highly expressed. Table 4 shows DAE of *UTMP* at SNP 1296 in these tissues, as measured by allelic ratios of G/A. The *UTMP* gene displayed DAE in all heterozygous individuals examined. Allele G seemed to be preferentially expressed in all endometrium tissues, although no formal statistical test was performed to evaluate the significance of this result. Extreme DAE was observed for 2 of 3 ovary

tissues (monoallelic expression) and for 2 of 4 caruncle tissues (G/A allelic ratios of 2.50 and 4.00).

DISCUSSION

In this study we report the expression pattern of the bovine *UTMP* gene in fetal and adult tissues and the association with production traits in 2 independent dairy cattle populations. The *UTMP* gene was chosen for this study because of its possible roles in health traits and previous results showing an association of the *UTMP* region with milk production and PL in dairy cattle.

This is the first report on expression analysis of *UTMP* transcripts in a wide range of cattle tissues. Expression of *UTMP* was examined in a total of 198 tissues obtained from 10 fetuses and 17 pregnant cows. Transcripts of *UTMP* were found in all cotyledon tissues examined and to a lesser extent in ovary, pituitary, and spleen fetal tissues. The *UTMP* transcripts were not

Table 3. Estimates of the allele substitution effect (of allele G) and standard errors for production and health traits in the CDDR and UW populations¹

Trait	CDDR		UW	
	Estimate (SE)	P-value	Estimate (SE)	P-value
Fat yield	-0.188 (1.894)	0.920	-0.081 (4.30)	0.985
Fat percentage	0.004 (0.008)	0.573	NR ²	
Milk yield	-33.14 (51.52)	0.520	-120.7 (115.2)	0.295
Protein yield	-1.520 (1.290)	0.237	-2.093 (3.045)	0.492
Protein percentage	-0.002 (0.016)	0.529	NR	
SCS	-0.016 (0.014)	0.236	0.065 (0.067)	0.336
Productive life	0.260 (0.098)	0.008	1.443 (0.675)	0.033

¹Populations: CDDR = Cooperative Dairy DNA Repository; UW resource population = University of Wisconsin resource population.

²NR = no records available for these traits.

Table 4. Expression ratios of G/A alleles at single nucleotide polymorphism 1296 of the uterine milk protein gene (*UTMP*) in 5 heterozygous individuals for ovary, endometrium, and caruncle tissues

Cow	Allelic ratio		
	Ovary	Endometrium	Caruncle
M2	G ¹	1.96	ND ²
M8	ND	1.27	0.51
M12	G ¹	1.78	4.00
M16	ND	1.81	1.56
M17	0.50	1.81	2.30

¹Monoallelic expression of allele G.²ND = not done.

found in any other fetal tissues examined. To exclude the possibility of RT-PCR reaction failure in tissues where *UTMP* expression was not observed, the β -actin gene was amplified for all tissues. Expression of the β -actin gene was detected in all examined tissues. However, we cannot exclude the possibility of variable expression of β -actin among the different tissues or individuals examined. Although the expression of *UTMP* in fetal tissues was surprising, its presence in reproductive tissues (cotyledon and ovary) might shed more light on the role of this gene in maintenance of pregnancy and fetal survival. Roberts and Bazer (1988) suggested that nutrition of the conceptus, growth control, and immunosuppression of the local maternal system are possible functions of the *UTMP*.

The expression of *UTMP* has been mainly studied in sheep, with only a few studies on expression and localization of the bovine gene. In sheep, *UTMP* is the predominant protein present in the uterine fluid from d 30 of pregnancy until term (Moffatt et al., 1987); *UTMP* mRNA is not detected in endometrium until d 14 of pregnancy (Ing and Roberts, 1989). In addition, mRNA levels of ovine *UTMP* have been found to be affected by day of pregnancy (Stewart et al., 2000).

In this study, bovine *UTMP* was predominantly expressed in endometrium, ovary, and caruncle tissues in pregnant cows. Inconsistent amplification of RT-PCR products was observed in oocytes, lung, liver, and spleen tissues. On the other hand, heart, muscle, and pancreas tissues did not express *UTMP* in any examined individual. Thus, the predominant expression of *UTMP* in reproductive tissues is consistent with an important role for this protein in reproductive success as previously suggested for the ovine gene (Ing and Roberts, 1989). However, in contrast to the aforementioned studies, this is the first report on expression of *UTMP* in tissues other than endometrium.

In this study, we aimed to investigate the association between *UTMP* and milk production traits and PL in dairy cattle. To do this, we identified 2 synonymous

SNP at positions 1179 (A/G) and 1296 (A/G) using the pooled DNA sequencing approach (Kwok et al., 1994; Khatib et al., 2006). The SNP at position 1296 was associated with a significant increase in PL. This finding parallels results previously obtained for the *PI* locus, which was also found to be associated with PL in dairy cattle (Heyen et al., 1999; Khatib et al., 2005). Taken together, these results provide strong evidence that the *UTMP* region is associated with the PL trait in dairy cattle. Unfortunately, these studies are not able to determine whether the effect observed is due to polymorphism in *PI*, *UTMP*, or another locus in that region. Additional research on this topic should be undertaken.

There is accumulating evidence that allelic variation in gene expression is correlated with phenotypic variability of quantitative and qualitative traits. In humans, only a few studies have addressed the possible roles of allele-specific expression in phenotypic variability among individuals and in disease susceptibility (Duno et al., 2004; Hirota et al., 2004; Murakami et al., 2004). For example, Duno et al. (2004) examined DAE of the *CLCN1* gene, which causes myotonia congenita, an inherited disorder characterized by delayed skeletal muscle relaxation. Interestingly, they found that the expression level of the mutant allele was higher than that of the wild-type allele, and they suggested that differential expression of *CLCN1* alleles might have roles in progression of myotonia congenital disease.

In this study, a polymorphism-based approach was used to analyze the allelic variation in expression of the bovine *UTMP* gene in reproductive tissues obtained from 9 heterozygous individuals. Reverse transcription-PCR analysis revealed that *UTMP* is expressed mainly in ovary, endometrium, and caruncle tissues. The DAE of *UTMP* in these tissues was measured as an allelic ratio of G/A alleles at SNP 1296. All heterozygous individuals examined displayed DAE, with allele G preferentially expressed in all endometrium tissues. Monoallelic expression (allele G) was observed in 2 of 3 ovary tissues and in 2 of 4 caruncle tissues. It is generally believed that genes displaying DAE would cause phenotypic variability across individuals. Given that *UTMP* has possible roles in health traits and based on the constant preferential expression of G allele at SNP 1296, it is plausible that this SNP is linked to improved PL.

Thus, the DAE of the bovine *UTMP* gene observed in this study is consistent with other studies on different genes that have also shown a correspondence between allelic variation in gene expression and phenotypic variability.

In summary, in this study we report that *UTMP* is primarily expressed in the bovine endometrium, ovary,

and caruncle tissues. Also, we report that *UTMP* SNP 1296 shows significant association with PL in 2 independent Holstein cattle populations. This SNP could be exploited by marker-assisted selection for genetic improvement of PL within a breeding nucleus or commercial population; or by marker-assisted introgression for transferring desired alleles from a resource population to a commercial population.

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