

# Identification of Polymorphisms in the Enhancer Region of the Bovine Prolactin Gene

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## Story in Brief

Prolactin (PRL) stimulates mammary development and promotes the formation and action of the corpus luteum during the female reproductive cycle in some mammals. The compounds associated with fescue toxicosis depress the levels of PRL in cattle, reduce milk yield, and lower reproductive efficiency. Studies have shown that administering other hormones may alleviate these symptoms by raising PRL levels in *Bos indicus* (Brahman) but not *Bos taurus* (Angus) cattle. This research project attempts to locate polymorphisms in the enhancer region of the PRL gene and correlate this to Brahman and Angus parentages. Two polymorphisms were found at positions -1161 and -1286. Genomic DNA from 73 Angus, Brahman, and Angus/Brahman crossed cows was analyzed for these single nucleotide polymorphisms (SNPs). Both SNPs showed allelic frequency variation between the Brahman and Angus populations. On the polymorphism at -1286, Brahmans were the predominant carriers with fewer carriers in the Angus population. This SNP has an allele frequency of 0.507. The polymorphism at -1161 showed even more variation in that the Brahmans appear to be the only carriers of the allele, with a frequency of 0.137. Results demonstrate that the enhancer region of the bovine prolactin gene has sequence differences that appear to be associated with Brahman cattle.

## Introduction

Prolactin (PRL) is a protein hormone that stimulates production of milk proteins. Serum concentrations of prolactin vary considerably due to breed, seasonal, and environmental effects. Fescue toxicosis is associated with a decrease in serum concentrations of prolactin; however, that fescue effect is less in Brahman cattle. The enhancer element of a gene serves as an attachment region for transcription factors that augment or repress basal levels of transcription. This sequence starts from approximately 1.5 kilobases to 300 base pairs before the PRL coding region. Polymorphisms, changes in the DNA base sequence, in this enhancer region could potentially alter the binding of promoter or enhancer elements, thus affecting expression of PRL in cattle. In a preliminary study of 13 cows (seven Brahman, six Angus) we found that the PRL enhancer region had two single nucleotide polymorphisms (SNPs) of interest. Therefore, this study was designed to determine the distribution of the two SNPs within a herd of cattle that contained both *Bos indicus* (i.e. Brahman) and *Bos taurus* (i.e. Angus) cattle.

## Experimental Procedures

**Polymerase Chain Reaction (PCR).** Genomic DNA was obtained from 17 Brahman (BB), 12 Brahman/Angus (BA), 21 Angus/Brahman (AB), and 23 Angus (AA) cows. Based on the NCBI Nucleotide sequence X16641 of PRL, primers were designed to amplify a 500 base pair fragment from positions -892 to -1392. Primer +PRL 892 (AAGTCCCCATAAGCACACTTGG) and primer -PRL 1392 (CTAACTTTAGGGAGTTCATACTG) were synthesized and supplied by Sigma - Genosys (Saint Louis, Mo.). The conditions for all PCRs were: 1x Buffer, 1.5mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2 μM of each primer, 0.08 μl Biolase TAQ polymerase

(Biolase USA, Inc., Randolph, Mass.). Fifteen μl of this mixture was added to 5 μl of each cow's DNA at 20 ng/μl. Each well was covered with a protective layer of mineral oil, and the plate enclosed by a plastic cover. The PCR program used for this reaction consisted of an initial 205°F for 2 minutes, then 35 cycles of 205°F for 30 seconds, 122°F for 30 seconds, 154°F for 1 minute. The reaction was completed with 10 minutes at 154°F, and then held at 46°F. A small portion of the PCR product was analyzed on a 1% agarose gel to determine if the PCR products were present.

**Enzyme Digestion.** The SNPs of interest occurred at two distinct restriction enzyme sites. The first site was digested by *Xba* I (TCT AGA) and the second site was digested by *Hsp92*II (CATG). Restriction enzymes were obtained from Promega (Madison, Wis.) and used to digest the PCR products. Ten microliters of PCR fragments was digested with 10 μl of *Xba* I according to manufacturer's instructions for 2 hours at 99°F, and another 10 μl was digested with *Hsp92*II according to manufacturer's instructions for 2 hours at 99°F. After digestion, the *Xba* I samples were analyzed on a 1% agarose gel to identify fragment sizes. The *Hsp92*II samples were analyzed on a 1.65% synergel because of the close size differences between the fragments.

## Results and Discussion

**Single Nucleotide Polymorphisms.** The SNPs identified in the initial sequencing were tested on 93 DNA samples to assess the frequency of the polymorphisms. Of the 93 test samples 73 were successfully amplified and restriction digested with *Hsp92*II and *Xba* I. If the allele had the SNP at -1286 (TTTAGA), the *Xba* I did not digest at this designated site, but the original sequence would be digested (TCTAGA). If the allele had the SNP at -1161 (CGTG), the *Hsp92*II did not digest at this position; yet if the allele was CATG, the enzyme did digest. Because cows have two alleles for every

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gene, one on each chromosome, the digestion site could occur on both, one, or neither of the alleles possessed by the cow. The alleles that were digested by the restriction enzymes were coded as 'W' for *Xba* I and 'Y' for *Hsp92II*. Those alleles that were not digested by the enzymes were labeled as 'X' for *Xba* I and 'Z' for *Hsp92II*.

*Hsp92II*. For the -1161 SNP, samples were homozygously cut (alleles YY), or heterozygous for the cut (alleles YZ), but no samples were completely digested (alleles ZZ). Table 1 presents the allelic frequency of animals by breed composition, showing that breed did affect the distribution of *Hsp92II* alleles ( $P < 0.001$ ). Additional analysis showed that paternal breed had a significant affect on the distribution of alleles with Brahman sires associated with 80% of the YZ alleles. The maternal breed was not a significant source of variation of frequencies of this *Hsp92II* allele. The sequences that contained the Z allele were either crossbred or Brahman DNA samples. That distribution contributes to the low allele frequency of 0.137 for this population.

*Xba* I. For the -1286 SNP, samples were homozygously cut (alleles WW), heterozygous (alleles WX), or homozygous uncut (alleles XX). The allelic distributions are presented in Table 2. A significant dependence between breed composition and the allelic frequencies was found. Both maternal and paternal breed were important ( $P < 0.05$  maternal,  $P < 0.01$  paternal). The frequencies of alleles show a near perfect distribution between W and X.

*Allelic Interaction*. Polymorphism frequencies of both SNP sites analyzed together reveals a significant effect of breed composition (Table 3). Those interactions demonstrate that several possible allele combinations are not present in the population. One interesting omission is that the heterozygous *Hsp92II* alleles (YZ) did not combine with the homozygous undigested *Xba* I alleles (XX).

At the *Hsp92II* site, the Brahman-sired cows were more likely to be heterozygous for the SNP than those sired by Angus. That find-

ing suggests that the CGTG allele (Y) originated in the Brahman population. For the *Xba* I restriction site, there was variation in the frequency of alleles between the purebred Angus (AA) and the purebred Brahman (BB). In the AAs, only 8.7% of the samples were homozygous for the uncut allele (XX), while the BBs had 47% homozygous for the same allele. This indicates that the TTTAGA allele exists in the Angus population, but at a lower frequency than in the Brahman population.

Looking at the data from both enzymes, several patterns emerge. The only cows to be cut by both *Hsp92II* and *Xba* I were purebred Angus, while the Brahman influence provided the alleles for heterozygosity. It is also worth noting that there were no *Xba* I uncut samples (XX) that are *Hsp92II* heterozygous (YZ). Due to the proximity of these SNPs, which were only 125 base pairs apart, it is highly probable that their frequencies are not independent. Together these polymorphisms provide a genetic marker for the *Bos taurus* and *Bos indicus* subspecies on the enhancer region of the PRL gene.

## Implications

With further research, these single nucleotide polymorphisms could provide genetic markers for production and reproductive traits related to prolactin synthesis. These single nucleotide polymorphisms may account for differences in prolactin concentrations and milk production between Brahman and Angus cattle. The polymorphic regions, if linked to prolactin concentrations and/or function, could explain part of the advantage of using Angus x Brahman cows over either of the purebreds.

**Table 1. Allele frequencies of *Hsp 92II* restriction site by breed composition.**

Breed <sup>1</sup>	YY <sup>2</sup>	YZ	ZZ
AA	23	0	0
AB	17	4	0
BA	5	7	0
BB	8	9	0

<sup>1</sup> Breed designations are AA = purebred Angus; AB = sire was Angus, dam was Brahman; BA = sire was Brahman, dam was Angus; BB = purebred Brahman.

<sup>2</sup> Allele YY represents the samples that were homozygous for the restriction site allele (CATG), YZ represents the heterozygous cows, and ZZ allele represents the samples that were homozygous for the SNP (CGTG).

**Table 2. Allele frequencies of *Xba* I restriction site by breed composition.**

Breed <sup>1</sup>	WW <sup>2</sup>	WX	XX
AA	8	13	2
AB	1	19	1
BA	3	6	3
BB	1	8	8

<sup>1</sup> Breed designations are AA = purebred Angus; AB = sire was Angus, dam was Brahman; BA = sire was Brahman, dam was Angus; BB = purebred Brahman.

<sup>2</sup> Allele WW represents the samples that were homozygous for the restriction site allele (TCTAGA), WX represents the heterozygous cows, and XX allele represents the samples that were homozygous for the SNP (TTTAGA).

**Table 3. Allelic combinations based on breed composition.**

<i>Xba</i> I	WW <sup>1</sup>			WX			XX		
<i>Hsp</i> 92II	YY	YZ	ZZ	YY	YZ	ZZ	YY	YZ	ZZ
Breed <sup>2</sup>									
AA	8	0	0	13	0	0	2	0	0
AB	0	1	0	16	3	0	1	0	0
BA	0	3	0	2	4	0	3	0	0
BB	0	1	0	0	8	0	8	0	0

<sup>1</sup>Allele WW represents the samples that were homozygous for the restriction site allele (TCTAGA), WX represents the heterozygous cows, and XX allele represents the samples that were homozygous for the SNP (TTTAGA). Allele YY represents the samples that were homozygous for the restriction site allele (CATG), YZ represents the heterozygous cows, and ZZ allele represents the samples that were homozygous for the SNP (CGTG).

<sup>2</sup> Breed designations are AA = purebred Angus; AB = sire was Angus, dam was Brahman; BA = sire was Brahman, dam was Angus; BB = purebred Brahman.