Characterization of Diverse Cattle Breeds for a Microsatellite Genetic Marker of the Bovine Prolactin Gene

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ABSTRACT

Cattle (n = 456) were evaluated for a microsatellite genetic marker associated with the bovine prolactin gene. Breeds represented were Simbrah (n = 278), Simmental (n = 29), Santa Gertrudis (n = 71), American Breed (n = 12), Salers (n = 12), Parthenais (n = 5), and Hotlander (n = 49). Three marker alleles (designated as A, B, and C) were detected with five observed marker genotypes; no AC genotypes were observed. Chi-square analysis illustrated differences in genotype distributions between breeds (P < .01). Overall, the frequencies for the marker alleles were .054, .923, and .023 for the A, B, and C alleles, respectively. Cattle of the American and Parthenais breeds appeared to have different allele frequencies compared to other types. Animals of the Simbrah, Simmental, Santa Gertrudis and Hotlander breeds did not differ from Hardy-Weinberg Equilibrium expectations for this genetic marker. Molecular characterization of cattle such as this can provide insights into their phenotypic attributes.

KEYWORDS: cattle breeds, microsatellite marker, prolactin

The interest in identification of genes and the understanding of how genes affect specific traits in livestock have increased dramatically in recent years. Discoveries from the human genome project have provided insights into several molecular genetic mechanisms in farm animal species. A specific class of genetic marker called microsatellites (Litt and Luty, 1989; Weber and May, 1989), which refers to repetitive DNA sequences of two or three base pairs, has been used extensively in mammalian genetic research the past ten years. It has been believed that many different kinds of hormones, growth factors, cellular hormone receptors, and metabolic clearance of hormones are involved in growth of animals (Trenkle and Marple, 1983). Prolactin, placental lactogen and growth hormone are related to each other and belong to the same super gene family (Miller et al., 1981; Camper et al., 1984; Hallerman et al., 1987). These three hormones are related by amino acid sequence, immunochemistry, and partially overlapping biological functions (Miller et al., 1981). Many different factors including cAMP, glucocorticoids, dopamine, estrogens, epidermal growth factor, calcium, and thyrotropin-releasing hormone are involved in the expression of the prolactin gene (Camper et al., 1984).

Both prolactin and growth hormone should be important candidate genes for growth in cattle because of their control for mammary growth, lactogenesis and lactation (Haller-

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man et al., 1987; Cowan et al., 1989). Since both are involved in lactation, these genes can potentially be used as markers to identify genetic differences among animals for milk production. Cowan et al. (1990) reported that prolactin genetic polymorphisms are associated with milk production. The objective of this study was to characterize feedlot steers of diverse breeds for a previously published microsatellite marker of the bovine prolactin gene and to calculate allele and genotypic frequencies for the different breeds to gain insight into underlying molecular genetic diversity.

MATERIALS AND METHODS

Experimental animals

The majority of the cattle in this project were *Bos indicus-Bos taurus*. Resource beef cattle, in target progeny groups of 12 per sire, all came from producers in Texas. All calves were weaned at 6 to 8 months of age, and fed in commercial feedlots in Gruver and Lubbock, TX. Upon arrival, animals were vaccinated against common clostridia bacterial and bovine respiratory diseases. Each animal was individually weighed and ear-tagged. In addition, they were subjectively evaluated for temperament score, muscle score and breed type score on 1 to 5 scales. Specific management of the cattle was described previously by Brooks (1997).

Approximately 20 ml of blood was taken by jugular venipuncture from each animal upon arrival at the feedlot. Within 48 hours, white blood cells were isolated from whole blood samples. White blood cell samples were kept at -70° C until DNA was extracted.

Laboratory protocols

Bovine genomic DNA was extracted from leukocytes layer derived from whole blood samples on each animal by according to the procedures of Maniatis et al. (1982) or using QIAamp blood kits and tissue kits for rapid purification of genomic DNA (QIAGEN, 1996). The marker we used was based on the polymerase chain reaction (PCR) that amplifies minute quantities of DNA.

PCR primer sequences for this prolactin gene marker were obtained from published literature (Fries et al., 1993). Once the primers were synthesized by a commercial company, they were cold tested (non-radioactively labeled) to verify the ideal PCR conditions. After cold testing, radioactively labeled PCR was performed by direct labeling. The isotope ^{32}P was used for direct labeling of DNA fragments by its addition to the PCR reaction mix (in a ratio of 0.006μl of α-^{32}P-dCTP (10 mCi/ml) per 1 μl of PCR reaction) immediately prior to amplification.

Each PCR reaction contained 100 ng of genomic DNA as template, 5 pM of both forward and reverse primers, 200 μM each of dATP, dCTP, dGTP, and dTTP, 1-2 μM $MgCl_2$, 2.5 μl Taq 10X buffer (Promega), 1unit Taq polymerase (Promega), and enough double distilled water to produce a final volume of 25 ml. All reactions were overlaid with mineral oil to prevent evaporation. The PCR conditions were: (1) 95° C for 5 minutes for initial step, (2) 95° C for 30 seconds, 57° C for 30 seconds, 72° C for 1 minute for a total of 35 cycles for amplification step, and (3) 72° C for 10 minutes as final elongation step.

After PCR, 10 ml of sequencing gel-loading buffer was added to each reaction, and the samples were denatured for 5 minutes at 95° C. Three μl of each PCR reaction was electrophoresed on 8% denatured polyacrylamide gels, which were run for 2.5 hours to 3.5 hours at 85 Watts. Gels were blotted onto filter paper support, vacuum dried (25 minutes at 80° C), and exposed to autoradiography film (Fuji RX) for 36 to 48 hours at -20° C. Genotypes of animals were determined based on their location on the gel (due to their

size) by their mobility on the gel relative to a known DNA sequence standard.

Statistical Analysis

Chi-square tests were used to investigate distribution of marker genotypes across breeds and to determine if the genotypic frequencies within breeds fit expectated Hardy-Weinberg equilibrium (HWE) proportions. Two-way contingency tables were constructed for breed by genotype through SAS (1992) for its Chi-square test. Derivations of expected frequencies of genotypes based on HWE proportions and an example are shown in Table 1. Because two allele frequencies were estimated from six genotypes the degrees of freedom associated with this Chi-square goodness of fit test was three (6 – 2 parameters estimated – 1 = 3). Frequencies of the three respective marker alleles were calculated from the observed marker genotypes as follows:

$$\text{Allele frequency} = \frac{2 \times \text{number of homozygotes} + \text{number of heterozygotes}}{2 \times \text{total number of individuals}}$$

Table 1. Expectations of Hardy-Weinberg Equlibrium Genotypic Proportions at a gene with three alleles.

Allele	Theoretical allele frequency	Example allele frequency
A	p	.20
B	q	.60
C	r	.20

Expected genotypic frequencies based on theoretical allele frequencies

Genotype	AA	AB	AC	BB	BC	CC
Genotype frequency	p^2	2pq	2pr	q^2	2qr	r^2

Example of calculation of expected Hardy-Weinberg proportions from estimated allele frequencies

Genotype	AA	AB	AC	BB	BC	CC
Genotype frequency	.04	.24	.08	.36	.24	.04

RESULTS AND DISCUSSION

Total number of animals with observable marker genotypes were 456 across seven different breeds. Three different alleles were detected as initially reported by Fries et al. (1993). They were designated as allele A at 156 base pairs (bp) in length, allele B at 159

bp, and allele C at 162 bp. The results showed five of the six possible genotypes (AA, AB, AC, BB, BC, and CC); no AC individuals were observed. Genotypic frequencies and allelic frequencies were calculated across all animals, and across animals within each breed (Table 2).

Table 2. Genotypic and allelic frequencies of the prolactin microsatellite marker across all animal and within each breed.

		Genotype frequency						Allele frequency		
Breed	n	AA	AB	AC	BB	BC	CC	A	B	C
Total	456	.009	.090	0.0	.862	.033	.007	0.054	0.923	0.023
Simbrah	278	.011	.061	0.0	.885	.036	.007	0.041	0.933	0.025
Simmental	29	.000	.103	0.0	.862	.034	.000	0.052	0.931	0.017
Santa Gertrudis	71	.014	.197	0.0	.789	.000	.000	0.113	0.887	0.000
American	12	.000	.083	0.0	.583	.250	.083	0.042	0.750	0.208
Salers	12	.000	.000	0.0	1.000	.000	.000	0.000	1.000	0.000
Parthenais	5	.000	.400	0.0	.400	.200	.000	0.200	0.700	0.100
Hotlander	49	.000	.082	0.0	.918	.000	.000	0.041	0.959	0.000

Table 3. Characterization of breeds involved in study.

Breed	Origin	Percent *Bos indicus*	Composition
Simbrah	USA	37.5	Composite of 5/8 Simmental, 3/8 Brahman
Simmental	Switzerland	0	Established Swiss breed with large size, high milk production, quite muscular and lean
Santa Gertrudis	USA	37.5	Composite of 5/8 Shorthorn, 3/8 Brahman
American	USA	50.0	Composite of 1/2 Brahman, 1/8 Bison, 1/4 Charolais, 1/16 Hereford, 1/16 Shorthhorn
Salers	France	0	Established French breed, moderate to large mature size, high milk production, muscular and lean
Parthenais	France	0	Established French breed, very muscular and lean
Hotlander	USA	18.8	Composite of 1/4 Angus, 1/4 Senepol, 5/16 Simmental, and 3/16 Brahman

The vast majority of our resource cattle had BB genotype (86.2%) due to the very high frequency of the B allele. Animals with AA (n = 4; 0.9%) and CC (n = 3; 0.7%) genotypes were very scarce. Animals with AB and BC genotypes were intermediate in frequencies at 9.0% and 3.3%, respectively . Allelic frequencies for the overall set of animals were .053, .923, and .023 for the A, B, and C alleles, respectively. Chi-square analysis indicated that differences in genotypic frequencies did differ between breeds (x^2, $_{24\ df}$ = 60.56; $P < .001$). However, one caveat with this test is that for several cells, the expected number of individuals were zero due to low frequencies of the A and C alleles. Nonetheless, these statistical differences agree with phenotypic information about these breeds, which are briefly described in Table 3. The difference in allele frequencies involving the comparison of the American breed animals (bison influenced) and the very muscular, very lean Parthenais cattle appear to have a lower frequency of the B allele than the others. Furthermore, the Parthenais animals had as many AB individuals as BB, but the American breed cattle had more BC than AB individuals. However, there are very few animals of these two breeds represented in this data set. It is therefore possible that these animals are not representative of their breeds. Of the breeds represented here, the American cattle had the higher frequency for the C allele and the Parthenais had the highest frequency of the A allele. It is possible that genes close to this marker are under selective pressure. However, of the four breeds formally tested for Hardy-Weinberg Equilibrium expectations, none significantly differed from expected values.

The vast majority of our cattle were *Bos taurus-Bos indicus* hybrids. Bishop et al. (1994) calculated average heterozygosity for 369 genetic markers (various types) and indicated that *Bos taurus* x *Bos indicus* F_1 crosses were most heterozygous (74.7 % ± 1.5 %, $P < 0.001$) across all genes. Unfortunately, this marker is not useful for mapping performance traits in this group even though they are primarily *Bos taurus-Bos indicus*. Further molecular genetic research with *Bos taurus-Bos indicus* animals will be very interesting.

The comparisons of allele and genotypic frequencies in these cattle are potentially useful, however. MacHugh et al. (1998) reported that microsatellite markers can be a very effective tool to identify breed in cattle. They assigned and identified 4 British Isles breeds and the Swiss Simmental breed cattle by using only eight microsatellites, with an accuracy of breed identification over 99%. MacHugh et al. (1997) also performed research with microsatellite markers to verify the genetic relationships among different groups of cattle (twenty distinct populations from Africa, Europe and Asia) and to characterize the extent and pattern of zebu genetic introgression in African populations. Ten of the microsatellite markers displayed large allelic differences when comparing Bos taurus and zebu cattle populations. These 10 marker loci showed a single allele or group of alleles that were present at high frequencies in Asian zebu breeds, intermediate frequencies in crossbred African zebu populations, low frequencies in African Bos taurus populations, and either absent or present at very low frequencies in European Bos taurus populations.

One aspect not covered by our study was the determination of relationship between this prolactin marker and performance data because of the very high frequency of the B allele. Genetic markers useful for gene mapping of performance traits should have high heterozygsity levels, which this prolactin marker did not. Quite a bit of research has been done to reveal associations between DNA markers for prolactin and growth hormone with growth and carcass traits in cattle. Herring (1995) reported that growth hormone microsatellite marker genotype had a significant effect on kidney, pelvic and heart fat percentage and slaughter hip height. Tank et al. (1994) reported that the genotypes of growth hormone PCR-RFLP marker had significant effects on hot carcass weight, rib section, and

longissimus dorsi muscle. Rocha et al. (1992) reported growth hormone polymorphisms were related to birth weight and shoulder width at birth. This type of information is valuable for additional genetic characterization of domestic livestock and future development of marker assisted selection programs for livestock breeders. However, none of these researchers found a single genetic marker that is powerful enough to become a good selection tool for growth, development or carcass traits in cattle.

There is another point that should be considered; combinations of small effects from several different genes probably regulate most economically important traits. Breeds that drastically differ from one another, and family lines that drastically differ from one another should be targets for molecular genetic research because those with the most variation in performance have the best chance for genetic differences. Researchers and breeders with both performance and pedigree data should attempt to maintain DNA libraries of those animals so that future potentially useful genetic markers can be verified across other populations.

SUMMARY

The interest in identification of genes affecting economically important traits and study of the underlying molecular genetic differences in livestock have increased dramatically in recent years. We investigated a previously published microsatellite genetic marker associated with the bovine prolactin gene. Among 456 cattle representing seven breeds, three marker alleles (designated as A, B, and C) were detected with five observed marker genotypes; no AC genotypes were observed. Apparent differences in genotype distributions between breeds were observed. Overall, the frequencies for the marker alleles were .054, .923, and .023 for the A, B, and C alleles, respectively. Cattle of the American and Parthenais breeds appeared to have different allele frequencies compared to other types. Animals of the Simbrah, Simmental, Santa Gertrudis and Hotlander breeds did not differ from Hardy-Weinberg Equilibrium expectations for this genetic marker. Molecular characterization of cattle such as this can provide insights into understanding of their phenotypic differences.

REFERENCES

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, J. Yoo, and C. W. Beattie. 1994. A genetic linkage map for cattle. Genetics. 136:619.

Brooks, C. 1997. Growth promotant effects on performance, composition, and palatability of steers with known parentage. M.S. Thesis, Texas Tech University, Texas, USA.

Camper, S. A., D. N. Luck, Y. Yao, R. P. Woychik, R. G. Goodwin, R. H. Lyons, Jr., and F. M.

Rottman. 1984. Characterization of the bovine prolactin gene. DNA. 3:237.

Cowan, C. M., M. R. Dentine, R. L. Ax, and L. A. Schuler. 1989. Restriction fragment length polymorphisms associated with growth hormone and prolactin genes in Holstein bulls: evidence for a novel growth hormone allele. Animal Genetics. 20:157.

Cowan, C. M., M. R. Dentine, R. L. Ax, and L. A. Schuler. 1990. Structural variation around prolactin gene linked to quantitative traits in an elite holstein sire family. Theor. Appl. Genet. 79:577.

Fries, R., A. Eggen, and J. E. Womack. 1993. The bovine genome map. Mammalian Genome. 4:405-428.

Hallerman, E. M., A. Nave, Y. Kashi, Z. Holqer, M. Soller and F. Beckmann. 1987. Restriction fragment length polymorphisms in dairy and beef cattle at the growth hormone and prolactin loci. Animal Genetics. 18:213.

Herring, K. L. 1995. Mapping of genes using a bovine bac library to determine their effects on economically important traits in cattle. M.S. thesis. Texas A&M University, Texas, USA.

Litt, M., and J.A. Luty. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within cardiac-muscle actin gene. Am. J. Hum. Genet. 44:397.

MacHugh, D. E., M. D. Shriver, R. T. Loftus, P. Cunningham and D. G. Bradley. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146:1071.

MacHugh, D. E., R. T. Loftus, P. Cunningham, and D. G. Bradley. 1998. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. Animal Genetics. 29:333.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning:A laboratory manual, 1st edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Miller, W. L., D. Coit, J. D. Baxter, and J. A. Martial. 1981. Cloning of bovine prolactin cDNA and evolutionary imlications of its sequence. DNA. 1:37.

QIAGEN, 1996. QIAamp Blood Kit and QIAamp Tissue Kit Handbook. QIAGEN Inc. Hilden, Germany.

Rocha, J. L., J. F. Baker, J. E. Womakc, J. O. Sanders, and J. F. Taylor. 1992. Statistical associations between restriction fragment length polymorphisms and quantitative traits in beef cattle. J. Anim. Sci. 70:3360.

SAS, 1992. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, North Carolina, USA.

Tank, P. A., D. Pomp, M. K. Nielsen, R. J. Rasby, B. L. Gwartney, and C. R. Calkins. 1994. Associations of DNA markers with carcass and production traits in Angus sired calves. Oklahoma Agricultural Experiment Station. Animal Science Research Report. P.14.

Trenkle, A., and D. N. Marple. 1983. Growth and development of meat animals. J. Anim. Sci. 57:273.

Weber, J. L., and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44:388.