Genetics and Molecular Biology, 28, 1, 84-87 (2005)

www.sbg.org.br

Research Article

Analysis of restriction fragment length polymorphism in the kappa-casein gene related to weight expected progeny difference in Nellore cattle

Fernando H. Biase, Analia Del V. Garnero, Luiz A.F. Bezerra, Arthur J.M. Rosa, Raysildo B. Lôbo and Lúcia Martelli

Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Genética, Ribeirão Preto, SP, Brazil.

Abstract

Restriction fragment length polymorphism (RFLP) has been detected at the bovine kappa-casein *locus*. The polymorphism has been analyzed for its effects in cattle production, mostly for milk traits and even for maternal effect on pre-weaning weights. We used polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) to genotype 408 Nellore animals for the non-silent mutation (Thr/Ile136 and Asp/Ala148) that characterizes the A and B variants of the polymorphism and compared expected progeny difference (EPD) for a maternal effect on 120 and 210 days weights and direct EPD for 120, 210, 450 and 550 day weight between AA and AB animals. The EPD values were obtained from the University of São Paulo (Brazil) Nellore Cattle Breeding Program, which evaluated 266,272 animals in 2001. Analysis of Variance was used to compare weight expected progeny differences (EPDs) between animals genotyped as AA and AB. The A allele frequency was 0.911. Although the AA animals had higher weight EPDs than AB animals the differences were not statistically significant ($p > 0.05$).

Key words: bovine, weight, kappa-casein, genetic marker.

Received: September 26, 2003; Accepted: September 28, 2004.

Introduction

The use of DNA polymorphic markers allows the determination of individual genotypes at many *loci* and provides information on population parameters such as allele frequencies as well as improving selection by marker assisted selection. Medrano and Aguilar-Cordova (1990) identified a restriction fragment length polymorphism (RFLP) at the kappa-casein (*CSN3*) bovine *locus* and detected two alleles (A and B) which, according to Lin *et al*. (1992), differed by two amino acid substitutions, tyrosine/isoleucine at position 136 and asparagine/alanine at position 143 of the kappa-casein protein.

The known participation of kappa-casein in the composition of milk protein (Bordim *et al*., 2001) justifies investigations correlating this polymorphism with milk production and composition. Although Haenlein *et al*. (1987) found no differences in milk traits between kappa-casein genotypes there have been many analyses of the kappa-casein *CSN3* polymorphism or linked markers and their direct effects on milk production. For example, in Finnish Ayrshire cows the B allele has been associated with high protein content in the first lactation (Ikonen *et al*., 2001) while in Holstein-Frisian cows the same allele was found to affect protein composition without modifying the concentration of protein (Bobe *et al*., 1999). However, Ojala *et al*. (1997) demonstrated that the B allele alone did not have a positive effect on milk or protein production but, as compared to other haplotype combinations, the alpha, beta and kappa casein genotypes ABA1A2BB increased protein content and resulted in an increase milk yield of 86 kg for the first lactation. Bovenhuis *et al*. (1992) reported that dairy cattle with the BB genotypes produced less milk in kilograms but that the milk produced had 0.8% more protein and 5.28 kg less fat in the first lactation than AA animals, these authors having reviewed the association of *CSN3* polymorphisms with kilograms of milk produced per lactation, percentage fat and protein and kilograms of fat and protein per lactation demonstrated a divergence regarding genotypes and their association with these traits. Quantitative trait *loci* screening in bovine chromosome 6, where the casein genes are located, have identified markers related to milk fat and milk protein yield (Kühn *et al*., 1999) and percentage protein, milk yield and fat yield (Velmala *et al*., 1999).

Send correspondence to Lúcia Martelli. Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Genética, Av. Bandeirantes 3900, 14040-030 Ribeirão Preto, SP, Brazil. E-mail: lrmartel@rge.fmrp.usp.br.

The *CSN3* polymorphism has also been studied in association with the weight of beef cattle. Moody *et al*. (1996) analyzed the relation of the *CSN3* polymorphism with direct and maternal expected progeny difference (EPD) for growth in Hereford cattle and suggested that the alleles explained 15% of the variation in EPD for birth weight and 8% of the maternal EPD for 180-days weight gain from birth to weaning. In Nellore cattle, Faria *et al*. (1999) investigated the relationship between the *CSN3* polymorphism and calve-weight at 205 days and found no difference between AA and AB animals. Tambasco *et al*. (2003) have genotyped crossbred cattle (Aberdeen Angus x Nellore, Canchim x Nellore, Simmental x Nellore) and found no differences in the average daily weight-gain from birth to weaning and average daily weight-gain from weaning to yearling for AA, AB and BB genotypes.

Although the published data indicate that there is no correlation between the *CSN3* polymorphism and weight in Nellore cattle it is still important to improve the available information on the effect of this polymorphism on estimated weight breeding values. In the study described in this paper we investigated kappa-casein as a candidate gene due to its biological importance for milk traits and possible influence on the maternal effect on calf growth-traits. Our main goal was to assess the allele frequency of the kappa-casein polymorphism and analyze possible effects of the different genotypes on the expected progeny weight differences of Brazilian Nellore cattle.

Material and Methods

Population

We genotyped and analyzed 408 *Bos indicus* (Nellore) male and female cattle born into eight Brazilian herds between 1996 and 2001. The animals were the offspring of 69 sires but no family design was used in the analysis. All the animals are included in the 2001 Nellore Cattle Breeding Program (NCBP) of the University of São Paulo (São Paulo state, Brazil) that predicted the breeding value for 266,272 calves, cows and bulls from herds distributed over 12 Brazilian states.

Laboratory analyses

Blood samples were collected in sterile tubes containing EDTA anticoagulant and the DNA extracted according to the method of Sambrook *et al*. (1989). The kappa-casein *CSN3* polymorphism genotypes were determined as described by Medrano and Aguilar-Cordova (1990) using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and the *Hinf* I restriction enzyme.

Breeding value estimation

The breeding values of the 408 animals were obtained from the 2001 NCBP, the methods used to estimate the expected progeny difference (EPD) being briefly explained in the next paragraph.

The traits studied were the standardized weight (kg) at 120, 210, 450 and 550 days (coded W_{120}, W_{210}, W_{450} and W_{550}). Weights were measured every three months from birth to the twenty-first month and cows were also weighted at calving and at weaning. The standardized weights were calculated based on the weights prior to and after the day on which the animals were weighed. Covariance components, heritabilities and breeding values were estimated using the multiple traits derivative-free restricted maximum likelihood (MTDFREML) software (Boldman *et al*., 1995) applying the animal model. The expected progeny differences (EPDs) were calculated as half the breeding value.

To analyze the W_{120} and W_{210} traits and estimate maternal EPD ($MEPD_{120}$ and $MEPD_{210}$) and direct EPD ($DEPD_{120}$ and $DEPD_{210}$) for these traits the model included data on the contemporary group (birth year and month, sex, heard and feeding data) and age class of cows at calving (< 36 to 47; 48 to 59; 60 to 70; 71 to 119; >119 months) as fixed effects. The direct effect of calf, cow, permanent environment of the cow and the associated error were considered as random effects. The DEPD for W_{450} and W_{550} ($DEPD_{450}$ and $DEPD_{550}$) were estimated with the same fixed effects described above and the animal direct effect and the error were considered to be random variables. Each trait was analyzed separately.

The inbreeding coefficient (F) for the animals was calculated from the genealogy data using the multiple traits derivative free numerator relationship matrix (MTDFNRM) function which forms part of the MTDFREML software (Boldman *et al*., 1995).

Statistical analyses

Genotypic and allele frequencies were calculated as described by Falconer and Mackay (1996) while MEPD and DEPD data for the different genotypes were subjected to analysis of variance (ANOVAR) using the general linear model (GLM) from the Statistical Analysis Software (SAS Institute Inc., 2000). The statistical model used was: Y_{ij}: $\mu + G_i + e_{ij}$, where Y_{ij} is the EPD for each trait of the ij^{th} animal, μ is the general mean of each trait, G_i is the fixed effect of the i^{th} *CSN3* (*Hinf* I) genotype and e_{ij} is the random error effect associated to the ij^{th} observation.

Results

The genotype frequencies for 408 animals were 0.8235 for the AA genotype and 0.1764 for the AB genotype while the frequency of the A allele was 0.911. The frequencies were similar when the animals were separated by heard or by sire (data not shown). Homozygote animals had higher mean values of weight EPD values (Table 1) but

Table 1 - Weight (kg) expected progeny difference (EPD) values for a sample of 408 Brazilian Nellore cattle genotyped for the Kappa-casein CSN3 (*Hinf* I) polymorphism.

	Kappa-casein genotypes (mean weight EPD (kg) ± standard deviation)	
Trait (kg)*	AA (n = 336)	AB (n = 72)
Maternal		
$MEPD_{120}$	1.16 ± 0.86	1.06 ± 1.07
$MEPD_{210}$	1.70 ± 1.26	1.55 ± 1.57
Direct		
$DEPD_{120}$	2.72 ± 1.77	2.47 ± 1.84
$DEPD_{210}$	4.17 ± 2.57	3.87 ± 2.69
$DEPD_{450}$	6.09 ± 4.24	5.83 ± 4.33
$DEPD_{550}$	7.51 ± 5.04	6.41 ± 5.13

MEPD = Maternal EPD; DEPD = Direct EPD.

there were no significant differences ($p > 0.05$) in the EPD values between AA and AB k-casein genotypes (Table 2).

Discussion

The genotype frequencies presented here are in accordance with Kemenes *et al*. (1999) who found an A allele frequency of 0.91 for 63 unrelated Brazilian Nellore cattle. Our results also agree with those of Del Lama and Zago (1996), Faria *et al*. (1999) and Tambasco *et al*. (2000) who genotyped 64, 82 and 180 Brazilian Nellore animals, respectively. Kemenes *et al*. (1999) analyzed other zebu breeds and found that the A allele frequency was 0.93 for the Gyr breed and 0.92 for the Guzera breed, similar to the 0.911 found by us for Nellore cattle, indicating that there is a tendency towards a higher A allele frequency in Brazilian zebu breeds. The highest inbreeding coefficient (F) for our sample was 0.14, with 98.53% of animals having $F < 0.1$. This data, and the frequency observed by Kemenes *et al*. (1999), indicate that the higher frequency of the A allele in Brazilian zebu breeds has not been influenced by inbreeding effects.

As for most zebu breeds, excluding the Gyr breed, Nellore cattle have been selected for meat production. This intensive selection for weight gain could have indirectly influenced kappa-casein allele frequencies, as proposed by Del Lama and Zago (1996). However, further studies should be carried out with zebu animals selected for meat and milk production in order to compare the allele frequencies.

Moody *et al*. (1996) demonstrated that A allele substitution had a positive effect on the EPD for birth weight, MEPD for birth weight, DEPD for 180-day weight-gain from birth to weaning and MEPD for 180-day weight-gain from birth to weaning ($p < 0.01$). The kappa-casein genotypes explained 15% of the EPD variability for birth weight and 8% of the MEPD for 180-day weight-gain from birth to weaning. Their hypothesis was that the differences in MEPD for *CSN3* genotypes were probably due to differences in milk composition as well as milk yield. The AA genotype would provide better maternal conditions for the growth of calves and consequently higher pre-weaning weight. Our result are the first analysis of differences in weight EPD values between AA and AB kappa-casein *CSN3* (*Hinf* I) genotypes in Brazilian Nellore cattle, but although the AA genotypes showed higher mean weight EPD values the differences found were not statistically significant, suggesting that this polymorphism does not influence the estimated breeding value for the growth trait.

Despite the fact that we analyzed EPD values only, the results agree with those of Faria *et al*. (1999) and Tambasco *et al*. (2003) which compared weight data sets. Faria *et al*. (1999) genotyped 82 cows for the A and B *CSN3* polymorphisms and evaluated the effect of the different genotypes on the weaning weight of the progeny and found that the 205-day calve-weight in kg did not differ between AA and AB cows ($p > 0.05$). Tambasco *et al*. (2003) found that the *CSN3* (*Hinf* I) genotypes had no effect on the average daily weight-gain in kg from birth to weaning and average daily weight-gain in kg from weaning to yearling in crossed calves resulting from Nellore females crossed with Aberdeen Angus, Canchim or Simmental bulls.

The situation as to how much the kappa-casein gene or a group of linked chromosome 6 genes contribute to milk traits is unclear. Depending on the breed and population investigated, the B and A alleles have been found to affect both milk yield and the components of the milk (Bovenhuis *et al*., 1992; Moody *et al*., 1990). However, both our results and previously published reports indicate that the kappa-

Table 2 - Analysis of variance mean square data and probability of significance between expected progeny difference (EPD) values for AA and AB k-casein CSN3 (*Hinf* I) genotypes of a sample of 408 Brazilian Nellore cattle.

		Mean square values for each trait*					
Source	Degrees of freedom	$MEPD_{120}$	$MEPD_{210}$	$DEPD_{120}$	$DEPD_{210}$	$DEPD_{450}$	$DEPD_{550}$
Genotypes	1	0.709	1.53	4.364	6.402	6.781	77.164
Error	407	0.812	1.742	3.179	6.767	18.101	25.671
Pr > F		0.35	0.35	0.24	0.33	0.54	0.08

MEPD = Maternal EPD; DEPD = Direct EPD.

casein *CSN3* polymorphism has not influenced weight-gain in Brazilian Nellore herds, although the difference in the frequency of the A and B alleles means that it is difficult to make an accurate estimation of their influence on weight-gain in Brazilian Nellore cattle. It thus appears that experiments with structured families should be carried out to provide information on the effect of this polymorphism on weight breeding value in Brazilian Nellore cattle.

Acknowledgments

The authors thank the Brazilian agencies CAPES, PRONEX and FAPESP (proc.: 98/16573-8) for financial support and also the Breeders belonging to NCBP-USP.

References

SAS Instituto Inc. (2000) Statistical Analysis Software (CD-ROM), Version 8.1, SAS Institute Inc., Cary.

Bobe G, Beitz DC, Freeman AE and Lindberg GL (1999) Effect of milk protein genotypes on milk protein composition and its genetic parameter estimates. J Dairy Sci 82:2797-2804.

Boldman KG, Van Vleck LD, Kriese LM and Kachman S (1995) MTDFREML: User's Guide. Department of Agriculture/Agricultural Research Service, Lincoln, 112 pp.

Bovenhuis H, Van Arendonk JAM and Korver S (1992) Associations between milk protein polymorphisms and milk production traits. J Dairy Sci 75: 2549-2559.

Bordim G, Cordeiro Raposo F, de la Calle B and Rodrigues AR (2001) Identification and quantification of major bovine milk proteins by liquid chromatography. Journal of Cromatography A 928:63-76.

Del Lama SN and Zago MA (1996) Identification of the k-casein and b-lactoglobulin genotypes in Brazilian *Bos indicus* and *Bubalus bubalis* populations. Braz J of Genet 19:73-77.

Falconer DS and Mackay TFC (1996) Introduction to Quantitative Genetics. 4rd edition. Longman Scientific and Technical, New York, 464 pp.

Faria FJC, Guimarães SEF, Lima RMG, Mourão GB and Pinheiro LEL (1999) Análise de polimorfismos do gene da k-caseína em fêmeas da raça Nelore e efeito sobre o peso à desmama de suas progênies. Arq Bras Med Vet Zootec 51:377-382.

Haenlein GFW, Gonyon DS, Mather RE and Hines HC (1987) Association of bovine blood and milk polymorphisms with lactation traits. J of Dairy Sci 70:2599-2609.

Ikonen T, Bovenhuis H, Ojala M, Ruottinen O and Georges M (2001) Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. J Dairy Sci 84:507-514.

Kemenes PA, Regitano LCA, Rosa AJM, Paker IU, Razook AG, Figueiredo LA, Silva NA, Etchegaray MAL and Coutinho LL (1999) K-casein, b-lactoglobulin and growth hormone allele frequencies and genetic distances in Nelore, Gyr, Guzerá, Caracu, Charolais, Canchim and Santa Gertrudis cattle. Gen Mol Biol 22:539-541.

Kühn C, Freyer G, Weikard R, Goldammer T and Schwerim M (1999) Detection of QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6. Anim Genet 31:333-340.

Lin CY, Sabour MP and Lee AJ (1992) Direct typing of milk proteins as an aid for genetic improvement of dairy bulls and cows. Anim Breed Abst 60:1-10.

Medrano JF and Aguilar-Cordova E (1990) Genotyping of bovine k-casein loci following DNA sequence amplification. Biotechnology 8:144-146.

Moody DE, Pomp D, Newman S and MacNeil MD (1996) Characterization of DNA polymorphisms in three populations of Hereford cattle and their associations with growth and maternal EPD in line 1 Herefords. J Anim Sci 74:1784-1793.

Ojala M, Famula TR and Medrano JF (1997) Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. J Dairy Sci 80:1776-1785.

Sambrook J, Fritch EF and Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press, New York, 18.136 pp.

Tambasco DD, Alencar MM, Coutinho LL, Tambasco AJ, Tambasco MD and Regitano LCA (2000) Caracterização molecular de animais da raça Nelore utilizando microssatélites e genes candidatos. Rev Bras Zootec 29:1044-1049.

Tambasco DD, Paz CCP, Tambasco-Studart M, Pereira AP, Alencar MM, Freitas AR, Couninho LL, Packer IU and Regitano LCA (2003) Candidate genes for growth traits in beef cattle crosses Bos taurus x Bos indicus. J Anim Breed Genet 120:51-56.

Velmala RJ, Vilkki HJ, Elo KT, Koning DJ and Mäki-Tanila AV (1999) A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. Anim Genet 30:136-143.

Associate Editor: Pedro Franklin Barbosa