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Association of the BoLA-DRB3 Gene with Production Traits and Occurrence of Mastitis in Crossbred Cows

Tadele Mirkena[*1], *C. S. Nagaraja*[1], *S. M. Byre Gowda*[2] *and M. G. Govindaiah*[2]

[1] Department of Animal Breeding, Genetics & Biostatistics, Veterinary College, UAS, Hebbal, Bangalore 560 024, India

[2] Institute of Animal Health & Veterinary Biologicals, Hebbal, Bangalore 560 024, India

Abstract

The potential associations of BoLA-DRB3 gene with production traits and occurrence of mastitis were studied using PCR-RFLP technique. DNA was isolated from 47 crossbred cows. BsuRI and Csp6I restriction enzymes, which produced 7 and 12 different allelic patterns, respectively, were used to digest the PCR product. The association of allelic pattern of BsuRI on milk yield was significant at $P \leq 0.076$. Patterns 'g' and 'e' were associated with the highest and lowest lactation mean milk yield, respectively. For allelic patterns of Csp6I, the difference was significant at P <
0.03. Pattern 'e' was associated with low mean lactation milk yield. The association of BsuRI allelic pattern on mean lactation milk fat yield was not significant (P > 0.10). Allelic patterns of Csp6I approached significance (P = 0.138) for differences in milk fat yield. BsuRI fragment patterns differed significantly (P < 0.06) for mean lactation protein yield. There was no significant difference for the allelic patterns of Csp6I. No significant difference was detected among the animals for mean lactation SNF yield with both enzymes. Chi-square analysis revealed no significant difference for the occurrence of mastitis between the animals with different allelic variants detected by both enzymes.

Keywords: BoLA-DRB3 gene, BsuRI, Csp6I, crossbred dairy cows, mastitis, PCR-RFLP, dairy production traits.

Introduction

The bovine lymphocyte antigen locus (BoLA), the major histocompatibility complex of cattle, has been mapped to chromosome 23 and consists of three classes, I, II and III (Bernoco *et al*., 1991; Anderson and Davies, 1994). The class II genes encode the immune associated molecules; they are found on the surfaces of B- and T-lymphocytes as well as macrophages and are glycoprotein heterodimers composed of an α- and β-chain, which are encoded by some tightly linked clusters of genes (Lewin, 2000). The class II region includes DQA, DQB, DRA, DRB, DOB, DYA, DYB, DNA and DIB genes (Anderson *et al*., 1988; Anderson and Rask, 1988; Stone and Muggli-Cockett, 1990). There are at least three DRB like genes in BoLA region, among which only the DRB3 gene is expressed to any considerable extent and is highly polymorphic (Anderson and Davies, 1994).

BoLA alleles have been associated with production traits such as milk, milk fat and milk protein yield (Sharif *et al*., 1998b; Starkenburg *et al*., 1997). Numerous associations between BoLA and incidence of health disorders have also been reported. For instance, associations of the bovine major histocompatibility complex with subclinical mastitis (Ostergard *et al.,* 1989; Lunden *et al.,* 1990); chronic posterior spinal paresis (Park *et al.,* 1993); dermatophilosis (Maillard *et al.,* 1996); foot and mouth disease (Becker, 1994; Haghparast *et al.,* 2000; Garcia *et al.,* 2001) and bovine respiratory cyncytial virus (Fogg *et al.,* 2001) have so far been reported.

* Corresponding author: Adami Tullu Agricultural Research Center, P. O. Box 178, Ziway, Ethiopia, E-Mail: tadelemkeba@yahoo.com; Fax: 251 6 41 32 30

Diseases that adversely affect milk production in dairy cows, especially diseases involving mammary health (e.g., mastitis), account for the largest reduction in producer income (Gill *et al*., 1990). Therefore, the possibility of using BoLA alleles as a selection tool to increase resistance to specific disease deserves careful consideration as it provides lasting solution. In this study, the associations of the BoLA-DRB3 gene with production traits (lactation milk, milk fat, milk protein and SNF yield) and susceptibility to mastitis were studied using PCR-RFLP technique.

Materials and Methods

Animals

Forty-seven animals comprising 10 Holstein Friesian, 24 Jersey and 13 Red Dane crossbred cows maintained at the University of Agricultural Sciences (UAS) Dairy Farm and Karnataka State Cattle Breeding Farm, Department of Animal Husbandry and Veterinary Services, Hessarghatta were used for the present study.

BoLA Class II Determination

Genomic DNA was prepared from whole blood of cattle using the high salt procedure of Miller *et al*. (1988). BoLA class II alleles were determined by PCR-RFLP technique as described by van Ejik *et al*. (1992) with minor modifications. Briefly PCR was conducted in Eppendorf Master Cycler (Germany) in a final volume of 50 µl PCR reaction mixture. Each amplification reaction consisted of 100 ng genomic DNA, 20 p.mole of each primer (P_2 and P_3), 100 mM of each dNTPs, 0.75 unit of *Taq* polymerase, and 5 µl of 10X assay buffer with 15 mM $MgCl_2$. Approximately a 450 bp of PCR product was amplified. This was digested with two restriction enzymes (*Bsu*RI/*Hae*III or *Csp*6I/*Rsa*I). Each digestion mixture consisted of 1X assay buffer, 20 units of the restriction enzyme and 10 µl of PCR product. The content was mixed thoroughly and digestion was carried out at 37^{O}C for one hour in an incubator. The digested product was run on 3 per cent agarose gel along with standard DNA marker (PhiX 174 DNA/HaeIII digest, Bangalore Genei, Ltd.), viewed under UV light and photographed.

Milk Composition Estimation

Gerber method and formal titration (Pyne's method) were employed for milk fat and protein estimation, respectively. Solids-Nonfat was estimated as:

$SNF = \frac{LR}{} + 0.2F_4 + 0.4$, where LR is the lactometer reading and F is the fat percentage.

Production and Health Records

One lactation milk yield data and health (incidence of mastitis) record of each of the cows selected for blood sampling were collected from respective farms. The milk yield data were adjusted to 305-days-mature equivalent basis. Fat, protein and SNF yields were computed from the milk yield data using the milk composition per cent obtained for each trait during the milk composition test.

Statistical Analysis

Test of significance for the associations of different allelic patterns with production parameters, viz., milk, fat, protein and SNF yield, were computed using a one-way analysis of variance of the Minitab statistical software (Minitab, 1996); $P \leq 0.10$ was reported significant. Tukey's pair-wise mean comparison technique was used to compare the different mean groups when the analysis of variance revealed significant difference between the different allelic patterns. In all analyses, alleles with a frequency less than 0.06 were

pooled in the 'other' category, except for fat, protein and SNF yield difference detected with *Csp*6I enzyme. Chi-square was used to test for significant association between BoLA-DRB3 gene and incidence of mastitis.

Results and Discussion

Association of DRB3 Gene with Production Parameters

Digestion of PCR products with *Bsu*RI and *Csp*6I restriction enzymes produced seven and 12 different allelic patterns, respectively. Table 1 summarizes the mean ± SE milk yield with different allelic patterns of *Bsu*RI and *Csp*6I enzymes. A significant difference (P<0.076) was detected for the *Bsu*RI allelic patterns and their association with milk yield. Allelic pattern 'g' of *Bsu*RI was associated with the highest milk yield (3233.4 ± 546.63 kg), whereas pattern 'e' was associated with the lowest mean lactation milk yield (2188.8 ± 131.07 kg). A modest significant difference (P < 0.03) was observed in the mean lactation milk yield of cows with different allelic patterns detected by *Csp*6I enzyme. The 'other' category was associated with the highest milk yield (3158.9 ± 358.8 kg). Cows with five different allelic patterns ('a', 'h', 'i', 'j' and 'l') that had a frequency less than 0.06 were pooled in to this category. Yet a significant difference could be seen among the other groups if the 'other' category is omitted out. For instance, allelic pattern 'd' was associated with the highest milk yield (2681.7 ± 211.66 kg). On the other hand allelic pattern 'e' was associated with a significantly reduced mean lactation milk yield (1661 ± 124.35 kg).

Associations between increased lactation milk yield and BoLA-DRB3.2*11 (Starkenburg *et al*., 1997), allele *8 and increased 305-day milk yield (Sharif *et al*., 1998b) have been reported. BoLA-DRB3.2*22 and 26 were associated with decreased lactation milk yield (Sharif *et al*., 1998b; Starkenburg *et al*., 1997). Though the present study suggested possible association of milk yield with RFLP patterns, it need be confirmed with the extension of the present study with large number of animals.

There was no significant difference detected in the present study between the fat yield of cows as investigated with *Bsu*RI and *Csp*6I enzymes (Table 2). However, cows with allelic pattern 'g' of *Bsu*RI had the highest fat yield of 164.21 ± 21.4 kg, whereas allelic patterns 'b' and 'e' both had low fat yield of 113.27 ± 20.44 kg and 111.42 ± 7.64 kg, respectively. Even though the analysis of variance revealed no significant difference between the allelic patterns of *Csp*6I for fat yield at P≤ 0.10, it approached significance at P = 0.138 for one-way analysis of variance. Cows with allelic pattern 'd' had the highest mean lactation fat yield of 131.4 ± 11.71 kg while those with allelic type 'g' had the lowest mean yield of 86.88 ±3.22 kg.

Even though significant difference could not be detected in the present study for the association of BoLA-DRB3 fragment patterns with milk fat yield, numerous associations of some alleles and the trait have been reported. Allele *24 (Starkenburg *et al*., 1997) and allele *8 (Sharif *et al*., 1998b) were reportedly associated with increased fat yield. On the other hand, Starkenburg *et al* (1997) and Sharif *et al* (1998b) reported the association of alleles *26 and *10 with reduced fat yield, respectively.

The allelic patterns of *Bsu*RI differed significantly (P < 0.06) for lactation protein yield. Allelic type 'g' was associated with the highest protein yield of 132.02 ± 25.7 kg and this figure was highly significantly different from the remaining mean values (Table 3). Allelic patterns 'b' and 'e' did not differ significantly for protein yield. There was no significant difference detected for the allelic variants of *Csp*6I at P < 0.10 (Table 3).

Table 1. Mean 305-days milk yield (kg) ± SE with different allelic patterns of *Bsu*RI and *Csp*61 enzymes (P ≤ 0.10).

***Bsu*RI[1]**			***Csp*61[2]**		
Allelic pattern	**N**	**Mean ± SE**	**Allelic pattern**	**N**	**Mean ± SE**
'b'	7	2734.6 ± 349.6[b]	'b'	3	2383.1 ± 691.83[e]
'e'	31	2188.8 ± 131.07[d]	'c'	4	2547.6 ± 454.7[c]
'g'	3	3233.4 ± 546.63[a]	'd'	7	2681.7 ± 211.66[b]
other	6	2715.5 ± 292.71[c]	'e'	7	1661.0 ± 124.35[h]
			'f'	6	2405.9 ± 305.53[d]
			'g'	5	2035.2 ± 185.19[g]
			'k'	3	2065.0 ± 358.93[f]
			other	9	3158.9 ± 358.8[a]

[1]Critical value = 3.78; CV = 29.87; SE = 118.44

[2]Critical value = 4.55; CV = 32.75; SE = 117.01

The different superscripts within a column indicate significant difference between the mean values.

In a study of Starkenburg *et al.* (1997), allele *7 was associated with significantly increased protein yield; alleles *8 and *26 were associated with reduced protein yield. According to Sharif *et al.* (1998b) allele *22 was also associated with reduced protein yield.

Animals with *Bsu*RI allelic variants did not differ significantly for mean SNF yield. However, type 'g' was associated with high SNF yield of 306.77 ± 58.84 kg, whereas type 'e' was associated with low SNF yield (Table 4). Allelic patterns of *Csp*6I approached significance at P < 0.133. Type 'd' was associated with high SNF yield of 230.72 ± 22.25 kg (Table 4). Type 'e' was associated with low SNF yield (148.99 ± 14.17 kg).

Table 2. Mean 305-days fat yield (kg) ± SE with different allelic patterns of *Bsu*RI and *Csp*6I enzymes (P ≤0.10).

***Bsu*RI[1]**			***Csp*61[2]**		
Allelic Pattern	**N**	**Mean ± SE**	**Allelic Pattern**	**N**	**Mean ± SE**
'b'	6	113.27 ± 20.44	'd'	6	131.4 ± 12.71
'e'	19	111.42 ± 7.64	'e'	5	95.17 ± 12.56
'g'	3	164.21 ± 21.4	'f'	4	109.0 ± 19.09
Other	5	123.29 ± 12.98	'g'	3	86.88 ± 3.22

[1]NS (Non significant); CV = 28.52; SE = 6.35

[2]NS; CV = 27.38; SE = 6.81

Elsewhere, there is no report on the association of DRB3 alleles with SNF. It is likely that alleles associated with milk, milk fat and protein yield may influence SNF yield too. In the present study *Bsu*RI allelic pattern 'g' was consistently associated with high milk, milk fat, protein and SNF yield. Similarly, *Csp*6I allelic type 'd' was associated with high lactation yield of the traits studied.

Association of DRB3 Gene with Susceptibility to Mastitis

The mastitis incidence data were analyzed using a Chi-square statistical technique. Here too, allelic patterns with a frequency of less than 0.06 were pooled in the 'other' category. The results of the analyses are given in Table 5. In the present study no significant association was detected between incidence of mastitis and the different allelic patterns revealed with the two enzymes although 61.7 per cent of the animals, which were

randomly distributed among the different allelic types, were being reported mastitis prone (i.e. have had mastitis at least once).

Table 3. Mean 305-days protein yield (kg) ± SE with different allelic patterns of *Bsu*RI and *Csp*6I enzymes (P ≤ 0.10).

***Bsu*RI**[1]			***Csp*61**[2]		
Allelic Pattern	**N**	**Mean ± SE**	**Allelic Pattern**	**N**	**Mean ± SE**
'b'	6	83.74 ± 12.12[c]	'd'	6	91.59 ± 7.43
'e'	19	80.94 ± 6.67[c]	'e'	5	64.36 ± 7.25
'g'	3	132.02 ± 25.7[a]	'f'	4	78.11 ± 17.41
Other	5	92.18 ± 8.56[b]	'g'	3	67.34 ± 5.2

[1]Critical value = 3.85; CV = 30.26; SE = 5.12

[2]NS; CV = 28.62; SE = 5.08

The different superscripts within a column indicate significant difference between the mean values.

Due to lack of proper recording on health performance of the animals, it was not possible to test for significant difference with respect to severity of mastitis. Sharif *et al*. (1998a) found no effects of BoLA-DRB3.2 alleles on somatic cell score (SCS) in Jersey cows but they indicated that allele *16 was significantly associated with lower SCS in Holsteins. The present study is in agreement with their investigation that BoLA alleles did not have significant associations with incidence of mastitis. However, others reported significant association between BoLA alleles and SCS, clinical mastitis score and increased incidence of mastitis. For instance, allele 23 was associated with reduced clinical mastitis score, allele 24 with highly significantly increased incidence of mastitis caused by major pathogens and allele 16 was associated with increased somatic cell score (Kelm *et al*., 1997; Starkenburg *et al*., 1997).

Table 4. Mean 305-days SNF yield (kg) ± SE with different allelic patterns of *Bsu*RI and *Csp*6I enzymes (P ≤ 0.10).

***Bsu*RI**[1]			***Csp*6I**[2]		
Allelic Pattern	**N**	**Mean ± SE**	**Allelic Pattern**	**N**	**Mean ± SE**
'b'	6	223.88 ± 32.19	'd'	6	230.72 ± 22.25
'e'	19	205.77 ± 18.39	'e'	5	148.99 ± 14.17
'g'	3	306.77 ± 58.84	'f'	4	210.50 ± 43.71
Other	5	244.25 ± 23.63	'g'	3	176.24 ± 13.15

[1]; CV = 32.02; SE = 13.66

[2]; CV = 28.87; SE = 13.03

Table 5. χ^2- test of significance of the association of different allelic patterns of *Bsu*RI and *Csp*6I enzymes with susceptibility to mastitis (P < 0.05).

BsuRI			**Csp6I**		
Allelic pattern	**χ^2**	**Significance**	**Allelic pattern**	**χ^2**	**Significance**
'b	0.072	NS	'b'	2.22	NS
'e'	0.0065	NS	'c'	0.15	NS
'g'	1.09	NS	'd'	0.524	NS
Other	1.36	NS	'e'	0.524	NS
			'f'	1.906	NS
			'g'	0.0019	NS
			'k'	0.9906	NS
			Other	3.105	NS

The reports on the association of mastitis susceptibility with BoLA-DRB3.2 alleles are inconsistent. More studies are necessary to determine whether genetic markers such as MHC genes control genetic resistance to mastitis. To determine causation of either resistance or susceptibility, cows that are free of mastitis can be genetically selected for the marker and subsequently challenged with a mastitis pathogen. It should then be possible to determine whether cows with a particular marker are more resistant to mastitis than cows without the marker.

BoLA-DRB3 alleles are associated with production traits in cattle and hence can serve as an important tool in marker-assisted selection. Regarding the association of MHC genes (BoLA-DRB3) with incidence of mastitis, further investigation with larger number of animals need be done so as to explore the existence of association between the two.

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