Detection of bovine leukocyte antigen DRB3 alleles as candidate markers for clinical mastitis resistance in Holstein × Zebu

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Detection of bovine leukocyte antigen DRB3 alleles as candidate markers for clinical mastitis resistance in Holstein × Zebu[1]

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ABSTRACT: Bovine leukocyte antigen DRB3 alleles from Holstein × Zebu crossbred dairy cows (n = 409) were analyzed using the PCR-RFLP technique. Exon II of DRB3 was amplified using locus-specific primers (HLO30/HLO32), followed by digestion with 3 restriction enzymes (*Rsa*I, *Bsty*I, and *Hae*III). Forty alleles were found with frequency ranging from 0.005 to 0.139. The most frequently detected alleles of Holstein × Zebu were DRB3*16, *51, *23, *11, *8, and *1, accounting for 61.12% of the alleles in the population. Detection of candidate alleles for clinical mastitis occurrence was performed by logistic regression. It was found that percentage of Holstein fraction in crossbred cows had a nonsignificant effect ($P > 0.05$). However, parity had a significant effect on mastitis occurrence. In addition, DRB3*1 and *52 were the most associated with the occurrence of clinical mastitis, whereas *15, *51, and *22 were associated with resistance in crossbred populations. This is the first report of association of DRB3*15 and *51 with mastitis resistance. The association was validated by examining the candidate alleles in another commercial population. Highly susceptible (n = 43) and resistant (n = 42) groups of Holstein × Zebu cows were investigated. The result confirmed that DRB3*1 and *52 could be considered as susceptibility alleles, whereas *15, *51, and *22 could be considered as resistant alleles in Holstein × Zebu raised under tropical conditions. In addition, allele effects on 305-d milk production were estimated by BLUP. It was shown that most alleles associated with high clinical mastitis occurrence were related to increased milk yield. This study revealed that allele DRB3*10 had the greatest effect on increasing milk yield with moderate resistance to clinical mastitis, which could be used as a potential marker for selection in dairy genetic evaluation.

Key words: bovine leukocyte antigen DRB3 allele, candidate marker, clinical mastitis, Holstein × Zebu

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INTRODUCTION

Crossbreeding between Holstein Friesian and Zebu with different levels of Holstein fraction has been used for dairy production in many tropical countries, such as Thailand, Vietnam, and the Philippines. Selection of dairy cows for mastitis resistance is a challenging concept for dairy producers and breeders, because mastitis is one of the most common diseases in dairy production. The incorporation of molecular genetic markers in the selection process through marker-assisted selection has an increased possibility for implementation (Stella et al., 2002; Dekkers, 2004; Schrooten et al., 2005). The PCR-RFLP technique revealed increased polymorphisms in exon II of DRB3 (Van Eijk et al., 1992; Gilliespie et al., 1999). Recently, more than 100 different alleles were investigated by PCR-RFLP (Van Eijk et al., 1992; Gelhaus et al., 1995; Maillard et al., 1999; do Nascimento et al., 2006; http://www.projects.roslin.ac.uk/bola/; last accessed Oct. 10, 2007) and PCR-sequence-base typing (Groenen et al., 1990; Russell et al., 1997). Although most of the mastitis occurrence could be accounted for by breed, parity, management, and environment (Dietz et al., 1997b; Kelm et al., 1997; Sharif et al., 1998), there have been several reports showing that bovine lymphocyte antigen (**BoLA**) DRB3, the major histocompatability complex, is associated with resistance to mastitis (Sharif et al., 1998; Kulberg et al., 2007; Rupp et al., 2007); DRB3.2 has also been observed to be associated with milk production traits

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Table 1. Data structure for the analyses

Item	Candidate herds	Validation herd
No. of herds	2	1
No. of parities	6	6
No. of Holstein fractions[1]	5	5
No. of animals with clinical mastitis records	409	85
305-d milk[2] (SD), kg	2,818 (1,620)	3,815 (1,488)
No. of sires[3]	74	15
Avg cows per sire	5.5	5.7

[1]Levels of Holstein fraction are grouped into 75 to 81.25%, >81.25 to 87.50%, >87.50 to 90.63%, >90.64 to 93.75%, and >93.75 to 96.88%.
[2]Average all parities.
[3]Two sires were shared between candidate and validation herds.

(Starkenburg et al., 1997; do Nascimento et al., 2006). The recent report by Rupp et al. (2007) showed that DRB3.2 was associated with antibody-mediated immune response, cell-mediated immune response, and somatic cell scores. Recent studies of the BoLA-DRB3.2 in dairy cattle have only involved purebreds (*Bos taurus* or *Bos indicus*). Additional studies on crossbreds are also required to assist dairy selection in tropical countries. The objectives of this study were: 1) to examine the polymorphisms of DRB3.2 alleles in a Holstein × Zebu population, 2) to investigate the candidate alleles associated with clinical mastitis occurrence and resistance, and 3) to estimate the effects of candidate alleles on milk production.

MATERIALS AND METHODS

Animals were handled and managed according to the Guidelines of Experimental Animal Care from the National Research Council of Thailand.

Animals and Data

To find candidate alleles, Holstein × Zebu dairy cows raised in 2 research locations (Roi Et Dairy Research Center, Khon Kaen University, and Lampaya Lkang Livestock and Breeding Research Center, Department of Livestock Development, Lopburi, Thailand) were used in the study. All cows were managed with similar feeding systems (separate roughage and concentrate feeding for lactating cows and grazing with no concentrate supplement for dry cows). Crossbreeding between Holstein and Zebu with grading up the fractions of Holstein has been applied in both herds. A total of 409 cows with mastitis data born from 1995 to 2005 were examined for DRB3.2 alleles by PCR-RFLP as described below. Mastitis was scored as a binary trait: 0 for no mastitis and 1 for 1 or more veterinary treatments for mastitis occurrence in first to sixth parity. Clinical illnesses and treatments were diagnosed by well-trained technicians.

To validate the candidate alleles in a different population, Holstein × Zebu dairy cows (n = 85) born from 1995 to 2003 on a commercial farm (Chokchai Dairy Farm, Nakorn Ratchasima, Thailand) were sampled. The crossbreeding and upgrading system between Holstein × Zebu was also used; however, an intensive corn silage feeding system was used in this herd. Two groups of mastitis-susceptible (n = 43) and resistant (n = 42) cows were selected and examined for particular alleles. In this study, cows with a history of mastitis treatment of more than 5 times during 1 to 3 parities were classified as susceptible, and cows with no mastitis treatment until the end of parity 3 were classified as resistant. Clinical mastitis was diagnosed by the farm veterinarian.

Seventy-four sires were used to produce 409 cows, and 15 sires were used to produce 85 cows in the candidate and validation herds, respectively (Table 1). The average number of cows per sire for all herds was 5.5 to 5.7. Two sires were shared between the candidate and validation herds.

To estimate the milk production effect, lactation records of multiparous cows from the 2 research herds were used in the analysis. Age at first calving had to be between 20 and 40 mo. Holstein fraction had to be between 75.00 and 96.88%.

Blood Collection and DNA Extraction

Approximately 10 mL of blood was collected from the tail vein of each cow into a vacutainer tube containing 10% of 0.5 *M* EDTA as anticoagulant. Genomic DNA was isolated from white blood cells using Puregene (Gentra Inc., Minneapolis, MN). Briefly, white blood cells were washed twice with 0.9% NaCl and centrifuged for 5 min at 595 × *g* at room temperature. Cell lysis buffer and protein precipitation buffer were added to the pellet. Cell lysate was then centrifuged for 15 min at 18,660 × *g* at 4°C. The supernatant was then transferred to a microtube, and absolute isopropanol was added. The DNA was precipitated at 18,660 × *g* for 15 min at 4°C. The supernatant was discarded, and the DNA pellet was washed 2 to 3 times with 75% ethanol. The DNA pellet was air-dried at room temperature and dissolved in DNA hydration buffer. Deoxyribonucleic acid quality and concentration were determined by UV spectroscopy. The DNA was diluted to 50 ng/μL as a working solution and stored at −20°C before using.

Amplification of BoLA-DRB3.2

The BoLA-DRB3 exon II was amplified by PCR using the single-step PCR modified from Gilliespie et al. (1999). Primers HLO30 (5′-ATC CTC TCT CTG CAG CAC ATT TCC-3′) and HLO32 (5′-TCG CCG CTG CAC AGT GAA ACT CTC-3′) were used. The reaction was carried out in a total volume of 20 μL containing the following: 100 ng of genomic DNA (2 μL of 50 ng/μL of genomic DNA), 1 × PCR buffer (2 μL of 10 × PCR buffer), 10 m*M* deoxynucleoside triphosphate (2 μL of 100 m*M* deoxynucleoside triphosphate), 5 m*M* $MgCl_2$ (2 μL of 50 m*M* $MgCl_2$), 0.5 m*M* of each primer (2 μL of 5 m*M* each primer), and 0.5 units of *Taq* DNA polymerase (Promega, San Diego, CA). Reactions were performed in a 96-well thermal cycler (GeneAmp PCR System 9600, Perkin-Elmer Applied Biosystems, Foster City, CA) according to the following cycling profile: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 64°C for 45 s, and 72°C for 45 s; and final extension at 72°C for 5 min.

Restriction of PCR Products

Polymerase chain reaction products from each cow were digested separately with 3 restriction endonucleases, *Rsa*I, *Hae*III, and *Bsty*I (New England Biolabs, Ipswich, MA) as described in Van Eijk et al. (1992). Each digestion reaction contained 5 μL of PCR products, 2 μL 10 × buffer, and 5 U of enzyme, in a total volume of 20 μL. Subsequently, each reaction was incubated at 37°C for *Rsa*I and *Hae*III and 60°C for *Bsty*I for at least 3 h.

PAGE and Genotyping of BoLA-DRB3

Deoxyribonucleic acid fragments were detected on a vertical electrophoresis unit (Mini-Protein III, Bio-Rad Laboratories, Richmond, CA) using a 12% denaturing polyacrylamide gel (Sigma Inc., St. Louis, MO) with 1 *M* TBE buffer (0.089 *M* Tris base, 0.089 *M* boric acid, and 0.002 *M* EDTA, pH 8.0). After electrophoresis at 300 mA for 45 min, gels were stained with GelStar (Gelstar Inc., Patchogue, NY) for 10 min. Deoxyribonucleic acid fragments were visualized by UV transillumination and photographed with the Syngene gel documentary system (Syngene Inc., Cambridge. IL). The BoLA-DRB3.2 alleles were identified by patterns described by Van Eijk et al. (1992) and reported on the BoLA nomenclature homepage (http://www.projects.roslin.ac.uk/bola/drb3pcr.html).

Detection of Candidate Markers

Allele frequencies were determined by $H_i = \sum n_i / N$, where H_i = the frequency for allele i; n_i = the number of allele i in a population; and N = the total number of alleles in the population. Data from alleles with frequency less than 2.5% were discarded before analyzing the association with clinical mastitis occurrence by logistic regression using SAS as described by Allison (1997). The statistical model was as follows:

$$ln\left(\frac{P_i}{1-P_i}\right) = \beta_0 + \beta_L X_L + \beta_H X_H + \beta_l X_l + \sum \beta_m BoLA_m + \varepsilon_{ijkl}, \quad [1]$$

where P_i = probability that cow i in parity j with level of Holstein fraction k raised in herd l was affected by at least 1 case of clinical mastitis occurrence (0 = no occurrence; 1 = occurrence); β_0 = the intercept; β_L = the regression coefficient for parity effect; β_H = the regression coefficient for level of Holstein fraction effect; β_l = a regression coefficient for herd effect; $\beta_1, \beta_2, \beta_3, \ldots \beta_m$ = the regression coefficients for allele 1, 2, 3,…m; X_L, X_H, X_l = the dummy variables for presentation of effects of parity, levels of Holstein fraction, and herd number; $X_1, X_2, \ldots X_m$ = the dummy variables for presentation of effects of allele 1, 2, 3,…m; and ε_{ijkl} = random error term.

The probability of mastitis occurrence $\left(\hat{P}_i\right)$ for each allele was estimated by:

$$\hat{P}_i = \frac{e^{\hat{\beta}_0 + \hat{\beta}_m}}{1 + e^{\hat{\beta}_0 + \hat{\beta}_m}}, \quad [2]$$

where $\hat{\beta}_0$ = the intercept; $\hat{\beta}_m$ = the regression coefficient for allele m estimated from [1]; and e = the exponential constant.

Estimation of Milk Yield Effects

Data for 305-d milk production was analyzed with a single-trait animal model as described by Starkenburg et al. (1997):

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Qm} + \boldsymbol{Za} + \boldsymbol{Wp} + \boldsymbol{\varepsilon},$$
$$Var\begin{bmatrix}\boldsymbol{a}\\ \boldsymbol{p}\\ \boldsymbol{e}\end{bmatrix} = \begin{bmatrix}\boldsymbol{A}\sigma_a^2 & 0 & 0\\ 0 & \boldsymbol{I}\sigma_p^2 & 0\\ 0 & 0 & \boldsymbol{I}\sigma_e^2\end{bmatrix},$$

where $\boldsymbol{y}$ = the vector of 305-d milk yield; $\boldsymbol{\beta}$ = a vector of fixed effects of contemporary group of month and year of calving, levels of Holstein fraction in crossbred cows, parity, and age at calving; $\boldsymbol{m}$ = the vector of fixed effects of DRB3.2 alleles, $\boldsymbol{a}$ = the vector of random additive genetic effects; $\boldsymbol{p}$ = the vector of random permanent environment effects; $\boldsymbol{\varepsilon}$ = the vector of random residual effects; $\boldsymbol{X}$, $\boldsymbol{Q}$, $\boldsymbol{Z}$, and $\boldsymbol{W}$ = the known incidence matrices, $\boldsymbol{A}$ = the additive numerator relationship matrix; and σ_a^2, σ_p^2, and σ_e^2 = the additive,

Table 2. Allele frequencies of BoLA-DRB3.2[1] (in parentheses) in 409 Holstein × Zebu crossbred cows typed by PCR-RFLP

Frequencies	Alleles
>0.10	*16 (0.139), *51 (0.122), *23 (0.117), *11 (0.103)
>0.05 to 0.10	*8 (0.076), *1 (0.054)
0.02 to 0.05	*52 (0.037), *27 (0.022), *32 (0.022), *naa (0.022), *22 (0.020)
0.02 to 0.01	*12 (0.017), *45 (0.015), *10 (0.012), *15 (0.012), *25 (0.012), *54 (0.012), *gad (0.012), *gbd (0.012), *iaa (0.012), *kaa (0.012), *kad (0.012), *nbd (0.012), *4 (0.010), *48 (0.010), *fed (0.010)
<0.01	*19 (0.007), *28 (0.007), *37 (0.007), *jda (0.007), *laa (0.007), *nad (0.007), *13 (0.005), *14 (0.005), *36 (0.005), *gae (0.005), *iba (0.005), *jba (0.005), *nab (0.005), *sed (0.005)

[1]Bovine leukocyte antigen DRB3.2 alleles were identified by patterns described by Van Eijk et al. (1992), Gelhaus et al. (1995), Maillard et al. (1999), Behl et al. (2007), and the BoLA nomenclature homepage (http://www.projects.roslin.ac.uk/bola/drb3pcr.html; last accessed Oct. 10, 2007).

permanent environment, and residual variances, respectively.

Heritability and repeatability were assumed to be 0.26 and 0.38 for 305-d milk yield based on the parameter estimates used for the national genetic evaluation program in Thailand. Best linear unbiased prediction was used to estimate breeding values and allele effects on 305-d milk yield. The analysis data had 851 lactation records from 409 cows, 642 animals in the pedigree, 31 groups of herd-year-month of calving, and 5 groups of Holstein fractions (50%, 50.1 to 75.0%, 75.1 to 87.5%, 87.5 to 93.75%, and >93.75%). The analyses were performed using the BLUPF90 programs in BLUPF90-DairyPAK 3.0 (M. Duangjinda; I. Misztal, University of Georgia; and S. Tsuruta, University of Georgia, unpublished data).

Sequence Alignment

All candidate alleles were sequenced using an ABI-377 Automated Sequencer (Perkin-Elmer Applied Biosystems). To avoid nucleotide mismatches, 2 sequences were analyzed twice: one with the forward primer and the other with the reverse primer. The alignment was performed by the IPD-MHC Database Alignment Tool (http://www.ebi.ac.uk/ipd/mhc/bola/align.html; last accessed Nov. 1, 2007).

RESULTS AND DISCUSSION

Allele Frequencies

Results showed that the BoLA-DRB3 locus was highly polymorphic in Holstein × Zebu cows. The distribution of the allele frequencies is shown in Table 2. Forty alleles were found with frequencies ranging from 0.005 to 0.139. Most alleles were similar to those previously reported in Taurine (Van Eijk et al., 1992; Gelhaus et al., 1995; Dietz et al., 1997b; Gilliespie et al., 1999; Maillard et al., 1999) and Zebu (do Nascimento et al., 2006; Behl et al., 2007) cattle. The remarkably high polymorphism found in the population might be influenced by allelic combination between Taurine and Zebu cattle. In addition, the Zebu foundation stock in Thailand comes from several types such as Brahman, Sahiwal, and Thai indigenous cattle. The most common

Table 3. Parameter estimates, SE, odds ratios, and probability of clinical mastitis occurrence from bovine leukocyte antigen DRB3 alleles

Variable	Parameter estimate	SE	Pr > χ^2	Odds ratio	Probability estimates[1]
Intercept	−1.0187	0.2764	0.0002	—	
DRB3*1	1.0011	0.4897	0.0409	2.7210	0.5387
DRB3*8	0.0543	0.3933	0.8901	1.0560	0.3118
DRB3*10	0.4396	0.6482	0.4977	1.5520	0.3998
DRB3*11	0.6307	0.3337	0.0588	1.8790	0.4464
DRB3*15	−2.4217	1.0769	0.0245	0.0890	0.0367
DRB3*16	0.0022	0.4073	0.9958	1.0020	0.3007
DRB3*22	−0.8644	0.8165	0.2898	0.4210	0.1531
DRB3*23	0.4153	0.3847	0.2804	1.5150	0.3939
DRB3*51	−0.7806	0.3978	0.0497	0.4580	0.1643
DRB3*52	0.9913	0.5776	0.0861	2.6950	0.5362
Parity	0.2266	0.0810	0.0052	1.2540	—
HF[2]	−0.0007	0.0028	0.7968	0.9990	—
Herd	0.2337	0.4312	0.8459	0.6890	—

[1]Probability of clinical mastitis occurrence at first lactation.
[2]HF represents level of Holstein fraction in crossbred cows.

Table 4. Allele frequencies for bovine leukocyte antigen DRB3.2 alleles of 85 Holstein crossbred cows typed by PCR-RFLP from susceptible (n = 43) and resistant groups (n = 42) on a commercial farm

	Alleles[1]								
Group	*51	*15	*22	*16	*8	*23	*1	*52	*Other[2]
Susceptible	—	—	—	0.023	0.047	0.209	0.047	0.302	0.372
Resistant	0.286	0.095	0.095	0.095	0.119	0.024	—	—	0.286

[1]Differences in allelic distribution between susceptible and resistant groups were tested by χ^2 test and were significant at $P < 0.001$.
[2]Susceptible group: *sdd (0.116), *41 (0.070), *37 (0.070), *naa (0.047), *nab (0.047), and *sba (0.023); resistant group: *gdd (0.119), *25 (0.048), *32 (0.048), *fad (0.048), and *kaa (0.024).

alleles found in Holstein × Zebu cows were DRB3*16, *51, *23, *11, *8, and *1, which accounted for 61.12% of the alleles in the population. These common alleles were similar to those in previous reports in purebred cows (Dietz et al., 1997b; Kelm et al., 1997; Sharif et al., 1998), except for DRB3*51, which was scarcely found in purebreds. It was found that DRB3*16 was the most frequent (0.139) allele in crossbred Holsteins in this study, whereas Gelhaus et al. (1995), Van Eijk et al. (1992), and Sharif et al. (1998) reported that DRB3*8 and *11 were the most frequently found alleles in purebred Holsteins. In this study, DRB3*8 and *11 were found in moderate frequencies. However, some reports show that DRB3*11 is absent in some breeds, such as Jersey (Sharif et al., 1998; Gilliespie et al., 1999) and Brazilian dairy Gir (da Mota et al., 2002).

Detection of Clinical Mastitis Occurrence

Table 3 shows the parameter estimates, SE, significant candidate alleles from logistic regression, and the estimated probability of clinical mastitis occurrence. Parity had a significant effect on mastitis occurrence ($P < 0.01$). Mammary tissue decomposition according to utilization and aging might be the reasons. In this study, however, the level of Holstein fraction in crossbred cows and herds had no significant effect ($P > 0.05$). It was found that DRB3*1 and *52 were the alleles most associated with clinical mastitis with probability of mastitis occurrence greater than 50%. It has been reported that DRB3*8 and *16 were the most susceptible alleles (Dietz et al., 1997b; Kelm et al., 1997; Sharif et al., 1998) with elevated somatic cell scores (Dietz et al., 1997a) in purebred Holsteins. However, this study showed that those alleles were associated with moderate susceptibility. In purebred Holsteins, it was found that DRB3*11 and *23 were associated with mastitis resistance (Dietz et al., 1997a,b; Kelm et al., 1997). The lack of agreement of the results of this study with previous studies may be due to several factors. Different populations may differ regarding linkage disequilibrium in the BoLA genes or in other genes that influence the immune response (do Nascimento et al., 2006). Another possibility is that different pathogens are present in the different environments, and the same allele may respond differently to alternative pathogens during various times of evolution. Pressure from rapidly evolving pathogens is largely responsible for generating and maintaining this diversity (Rupp et al., 2007), which may relate to the molecular structure and antigen-presenting capacity of this allele. The most resistant alleles with probability of mastitis occurrence less than 20% were DRB3*15, *22, and *51. Significant association of DRB3*15 and *51 with mastitis resistance was also found ($P < 0.05$). The association of DRB3*22 with mastitis resistance was similar to that found by Dietz et al. (1997b) and Kulberg et al. (2007); however, the effect was not significant in this study. The most susceptible alleles with an increased probability of mastitis occurrence and increased odds ratios were DRB3*1 and *52. This study also confirmed that breed structure could be affecting the association between DRB3 alleles and clinical mastitis occurrence. In purebred Holsteins, several alleles have been reported as resistant alleleles, such as DRB3*11 (Kelm et al., 1997; Kulberg et al., 2007; Rupp et al., 2007), *23 (Dietz et al., 1997a,b; Kelm et al., 1997), *8 (Dietz et al., 1997a,b), and *16 (Dietz et al., 1997a,b; Kelm et al., 1997; Sharif et al., 1998); however, they were not the most resistant alleles in the Holstein × Zebu cattle used in this study.

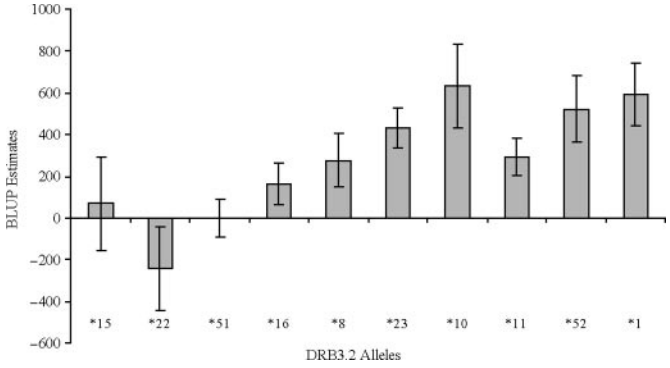

Figure 1. 305-d milk yield BLUP estimates for DRB3.2 alleles (alleles are listed from left to right in degree of mastitis susceptibility).

Validating Candidate Alleles

Table 4 shows that candidate alleles were only partially observed in the tested population. The most frequent alleles in the susceptible and resistant groups were DRB3*52 and *51, respectively. This result shows that DRB3*52 and *1, which were candidates for susceptible

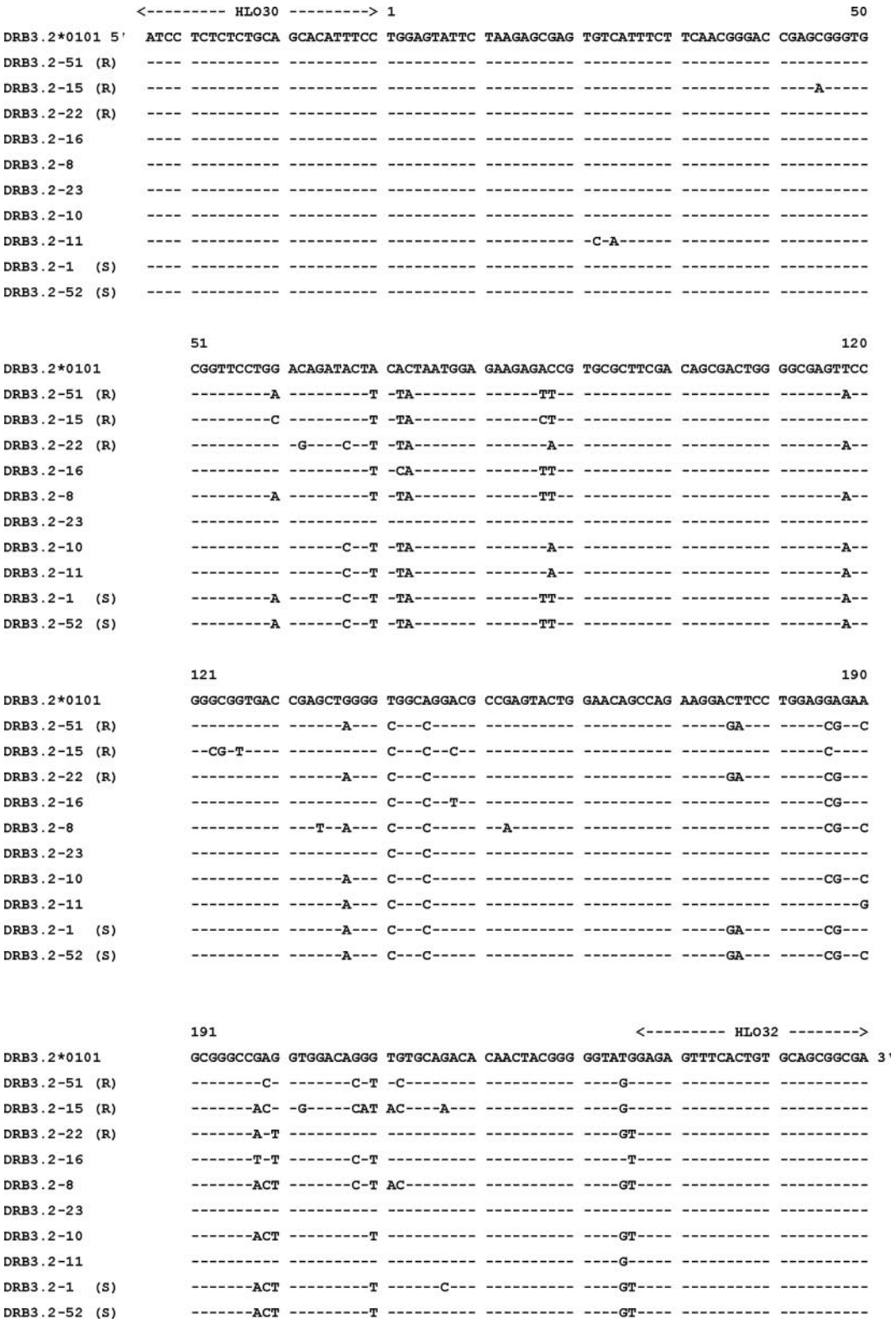

Figure 2. Alignment of the nucleotide sequence of 10 candidate alleles of bovine leukocyte antigen DRB3.2 for mastitis occurrence from this study. Dashed lines indicate sequence identity with respect to DRB3.2*0101 (accessed from European Bioinformatic Institute, http://www.ebi.ac.uk/ipd/mhc/bola; last accessed Nov. 1, 2007). HLO30/HLO32 located for forward and reversed primer binding sites. Alleles associated with clinical mastitis resistance or susceptibility in this study are marked as R and S, respectively. All sequences were submitted to National Center for Biotechnology Information GenBank with accession numbers EU58977 (DRB3.2*51), EU586800 (DRB3.2*15), EU586801 (DRB3.2*22), EU586802 (DRB3.2*16), EU586803 (DRB3.2*8), EU586804 (DRB3.2*23), EU586805 (DRB3.2*10), EU586806 (DRB3.2*11), EU586807 (DRB3.2*1), EU586808 (DRB3.2*52).

alleles, were found only in the susceptible group, and DRB*51, *15, and *22, which were candidates for resistant alleles, were found only in the resistant group. This result confirmed that DRB3*52 can be considered the most susceptible allele and DRB3*51 the most resistant allele in Holstein × Zebu cattle raised under tropical conditions. However, DRB3*16, *8, and *23 cannot be confirmed with certainty as either susceptible or resistant alleles, because they were found in both groups.

Estimation of Milk Yield Effects

The effect of DRB3.2 alleles on 305-d milk yield is shown in Figure 1. It was found that allele DRB3*10 had the greatest effect on increasing milk yield, which agrees with Starkenburg et al. (1997), who investigated purebred Holsteins. In addition, allele DRB3*22 was associated with decreasing milk production. Noticeably, the mastitis-susceptible associated alleles tended to be associated with increasing milk yield, whereas mastitis-resistant associated alleles tended to be associated with decreasing milk yield. However, this study reveals that DRB3*10 could be used as a candidate gene marker for improving milk production with moderate mastitis resistance.

Sequence Alignment

Nucleotide sequences of all candidate alleles between HLO32 and HLO30 with National Center for Biotechnology Information GenBank accession number are shown in Figure 2. Sequences of DRB*51, *15, and *22 (resistant alleles) were compared with DRB*1 and *52 (susceptible alleles). Five mutation points were found that could be used to distinguish between resistant and susceptible alleles. Specifically, mutation points were observed at positions 67 (A → C), 200 (G → T), 208 (C → G), 212 (C → G), and 236 (G → T).

This study identified alleles at the DRB3.2 locus with single-step PCR-RFLP, which was modified from the nested PCR described by Van Eijk et al. (1992), and considered estimation of gene substitution effects of the alleles. The most common alleles found in crossbreds were DRB3*11, DRB3*23, DRB3*51, and DRB3*16. Allele DRB3*51 was the most prevalent in Holstein × Zebu crossbreds. Mastitis occurrence was related to lactation number. However, the level of Holstein fraction in crossbred cows had no effect. The DRB3*1 and DRB3*52 alleles were the most associated with mastitis occurrence, whereas DRB3*15, DRB*22, and DRB3*51 were the alleles most associated with mastitis resistance. Candidate alleles were validated in another crossbred populations. It was confirmed that DRB3*1 and DRB3*52 are susceptible alleles and DRB3*51 and DRB3*15 are resistant alleles in crossbred Holsteins. Alignment of sequences showed that there were 5 important mutation points that could be used to differentiate between susceptible and resistant alleles. In addition, the mastitis-susceptible associated alleles tended to increase milk yield, whereas mastitis-resistant associated alleles tended to decrease milk yield. Allele DRB3*10 had the greatest effect on increasing milk yield with moderate resistance to clinical mastitis and could be used as a potential marker for selection in dairy genetic evaluation.

LITERATURE CITED

Allison, P. D. 1997. Survival Analysis Using the SAS System. A Practical Guide. SAS Inst. Inc., Cary, NC.

Behl, J. D., N. K. Verma, R. Behl, M. Mukesh, and S. P. S. Ahlawat. 2007. Characterization of genetic polymorphism of the bovine lymphocyte antigen DRB3.2 locus in Kankrej cattle (*Bos indicus*). J. Dairy Sci. 90:2997–3001.

da Mota, A. F., J. E. Gabriel, M. L. Martinez, and L. L. Coutinho. 2002. Distribution of bovine lymphocyte antigen (BoLA-DRB3) haplotypes in Brazilian dairy Gir cattle (*Bos indicus*). Eur. J. Immunogenet. 29:223–227.

Dekkers, J. C. M. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. J. Anim. Sci. 82 (E. Suppl.):E313–E318.

Dietz, A. B., N. D. Cohen, L. Timms, and M. E. Kehrli Jr. 1997a. Bovine lymphocyte antigen class II alleles as risk factors for high somatic cell counts in milk of lactating dairy cows. J. Dairy Sci. 80:406–412.

Dietz, A. B., J. C. Detilleux, A. E. Freeman, D. H. Kelley, J. R. Stable, and M. E. Kehrli Jr. 1997b. Genetic association of bovine lymphocyte antigen DRB3 haplotypes with immunological traits of Holstein Cattle. J. Dairy Sci. 80:400–405.

do Nascimento, C. S., M. A. Machado, M. L. Martinez, M. V. G. B. da Silva, M. F. M. Guimarães, A. L. Campos, A. L. S. Azevedo, R. L. Teodoro, R. D. S. Verneque, S. E. F. Guimarães, and D. A. A. Oliveira. 2006. Association of the bovine major histocompatibility complex (BoLA) BoLA-DRB3 gene with fat and protein production and somatic cell score in Brazilian Gyr dairy cattle (*Bos indicus*). Genet. Mol. Biol. 29:641–647.

Gelhaus, A., L. Schnittger, D. Mehlitz, R. D. Horstmann, and C. G. Meyer. 1995. Sequence and PCR-RFLP analysis of 14 novel BoLA-DRB3 haplotypes. Anim. Genet. 26:147–153.

Gilliespie, B. E., B. M. Jayarao, H. H. Dowlen, and S. P. Oliver. 1999. Analysis and frequency of bovine lymphocyte antigen DRB3.2 alleles in Jersey cows. J. Dairy Sci. 82:2049–2053.

Groenen, M. A. M., J. J. van der Poel, R. J. M. Dijkhof, and M. J. Giphart. 1990. The nucleotide sequence of bovine MHC class II DQB and DRB genes. Immunogenetics 31:37–44.

Kelm, S. C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstien cattle. J. Dairy Sci. 72:1767–1775.

Kulberg, S., B. Heringstad, O. A. Guttersrud, and I. Olsaker. 2007. Study on the association of BoLA-DRB3.2 alleles with clinical mastitis in Norwegian Red cows. J. Anim. Breed. Genet. 124:201–207.

Maillard, J. C., C. Renard, P. Chardon, I. Chantal, and A. Bensaid. 1999. Characterization of 18 new BoLA-DRB3 alleles. Anim. Genet. 30:200–203.

Rupp, R., A. Hernandez, and B. A. Mallard. 2007. Association of bovine leukocyte antigen (BoLA) DRB3.2 with immune response, mastitis, production and type traits in Canadian Holsteins. J. Dairy Sci. 90:1029–1038.

Russell, G. C., C. J. Davies, L. Andersson, S. Mikko, S. A. Ellis, E. J. Hensen, H. A. Lewin, N. E. Muggli-Cockett, and J. J. V. D. Poel. 1997. BoLA class II nucleotide sequences, 1996: Report of the ISAG BoLA nomenclature committee. Anim. Genet. 28:169–180.

Schrooten, C., H. Bovenhuis, J. A. M. van Arendonk, and P. Bijma. 2005. Genetic progress in multistage dairy cattle breeding schemes using genetic markers. J. Dairy Sci. 88:1569–1581.

Sharif, S., B. A. Mallard, B. N. Wilkie, J. M. Sargeant, H. M. Scott, J. C. Dekkers, and K. E. Leslie. 1998. Association of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) haplotypes with occurrence of disease and milk somatic cell score in Canadian dairy cattle. Anim. Genet. 29:185–193.

Starkenburg, R. J., L. B. Hansen, M. E. Kehrli Jr., and H. Chester-Jones. 1997. Frequencies and effects of alternative DRB3.2 alleles of bovine lymphocyte antigen for Holsteins in milk selection and control lines. J. Dairy Sci. 80:3411–3419.

Stella, A., M. M. Lohuis, G. Pagnacco, and G. B. Jansen. 2002. Strategies for continual application of marker-assisted selection in an open nucleus population. J. Dairy Sci. 85:2358–2367.

Van Eijk, M. J. T., J. A. Stewart-Haynes, and H. A. Lewin. 1992. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. Anim. Genet. 23:483–496.