



## Association of $\beta$ -Lactoglobulin Variants with Milk Yield and Composition in Dairy Cattle

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### Abstract

Major milk proteins have considerable variants which comes from substitution and deletions in their amino acid sequences. Variants in genes that code for milk proteins, such as  $\beta$ -lactoglobulin ( $\beta$ -LG) have been established as genetic markers for milk production and milk protein composition in dairy cattle. The effect of  $\beta$ -LG variant on milk production traits, such as milk yield, fat yield, protein yield, fat percentage and protein percentage, was estimated for 482 Holstein cows in the first lactation. The  $\beta$ -LG variants were determined by PCR-RFLP technique at the DNA level. Single trait linear model was used for the statistical analysis of the data. Results of this study indicated that  $\beta$ -LG variants affected significantly protein yield ( $p < 0.05$ ) and fat percentage ( $p < 0.05$ ). Animals with the AA variant produced 31kg of milk protein more than animals with the BB variant. On the contrary, cows with the BB variant had fat percentage higher by 0.35 and 0.32% compared with cows with the AA and AB variants, respectively. No associations between the  $\beta$ -LG variants and milk yield, protein percentage and fat yield were found. Therefore, milk production traits could be improved through  $\beta$ -LG typing by increasing the frequency of A variant for protein yield or the frequency of B variant for fat content in Holstein dairy cattle population.

**Key words :** milk protein,  $\beta$ -LG variant, DNA typing, milk production traits

### INTRODUCTION

Traditionally, fluid milk and other dairy products have been essential components of the human diet in a variety of cultures, mainly because of the high quality of the protein contained in milk and the variety of minerals and vitamins. Today, the dairy industry has the technological ability to produce large quantities of various dairy products. However, the dairy industry continually strives to improve the quality of its products. It has long been known that the manufacturing properties of milk are influenced by the relative composition of its proteins. The proportion of the protein components in milk shows individual variation because of environmental and genetic factors. Bovine major milk proteins show genetic polymorphism and it has been demonstrated that frequency distribution of the genetic variants are breed specific (Ng-

Kwai-Hang, 1998). The milk protein polymorphisms have been found to be associated with the differences in composition and processing qualities of milk and also linked to some of the production traits (Mitra *et al.*, 1998). Some milk protein variants have a significant effect on milk production traits and on cheesemaking properties and are therefore discussed as candidates in search for genetic marker for economically important milk production traits. Whey protein concentrates are used as food ingredients because of their functional properties as well as their nutritional properties (Creamer *et al.*, 2004).

The major protein of bovine whey is  $\beta$ -Lactoglobulin ( $\beta$ -LG), which is responsible for many of the functional properties of whey protein products (Mulvihill and Kinsella, 1987). Although no clear physiological function has been defined for  $\beta$ -LG, its binding affinity for retinal (Fugate and Song, 1980) and fatty acids (Frapin *et al.*, 1993) has been demonstrated, suggesting a possible role of  $\beta$ -LG in the transport and metabolism of these components. Bovine  $\beta$ -LG occurs naturally in a number of genetically different variants,

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which differ from one another by substitutions of one or more amino acids within the protein sequence (Eigel *et al.*, 1984). The most common variants are known as  $\beta$ -LG A and  $\beta$ -LG B. The effects of bovine  $\beta$ -LG variants on milk yield and composition (Bech and Kristiansen, 1990; Hill, 1993; Ng-Kwai-Hang *et al.*, 1986) and cheese-making properties (Alean-dri *et al.*, 1990; Lunden *et al.*, 1997; Marziali and Ng-Kwai-Hang, 1986; Schaar *et al.*, 1985) have been extensively studied. The genes that encode the major milk proteins are candidate genes for the observed variation in protein composition. It has been suggested that milk protein variants could be used routinely as additional selection criteria for dairy sires (Gibson *et al.*, 1990). However, depending on breed, population or region, the same genes and alleles were associated with different traits and effects. Although effects of milk protein polymorphisms on milk production traits have been investigated during the past decades, in some cases, results are still conflicting. The objectives of this study were to determine  $\beta$ -LG variant at the DNA level and to investigate their associations with milk yield and composition in Korean dairy cow population.

## MATERIALS AND METHODS

### Animals and Data

DNA samples were collected from 482 Holstein lactating cows in several commercial dairy farms (Sungwon, Hanil and Samyang farms) registered in the official performance testing program at Kyunggi and Kangwon do. The following 305-d milk production traits of cows in first lactation records were used in the statistical analyses: milk yield, fat percentage, fat production, protein percentage and protein production. Means and standard deviations of the milk production traits for the Holstein dairy cow population examined in this study are in Table 1.

### DNA Extraction from Milk Samples

3 ml of milk are diluted 1:1 in 50 mM Tris-HCl (pH 7.6) and centrifuged at 2,500rpm for 10 min. The cell pellet is resuspended in a washing solution (10 mM Tris-HCl, pH 7.5, 1mM EDTA, pH 8.0) and transferred to Eppendorf tubes. The cells are briefly centrifuged in microcentrifuge and the resulting cell pellet is resuspended in lysis buffer-K (10 mM Tris, pH 8.3, 50 mM KCl, 0.5 % Tween, 0.4% Proteinase K). The

**Table 1. Means and standard deviations of 305-d milk production traits from 482 first lactation cows**

Trait	$\bar{X}$	SD
Milk (kg)	7,763	1,460
Fat (%)	3.73	0.43
Protein (%)	3.12	0.17
Fat (kg)	285	42.5
Protein (kg)	249	32.4

suspension is incubated at 56°C for 45 min and subsequently at 95°C for 10 min. After ultra centrifugation briefly, the DNA samples were stored in the refrigerator.

### PCR-RFLP Analysis

For analysis of the  $\beta$ -LG variants, the DNA between nucleotides 367 and 629 of the  $\beta$ -LG gene was amplified by the use of the oligonucleotide primer sequences described by Chung *et al.*(1994): 5'-gTCCTTgTgCTgACACCgACTACA -3' and 5'-CAGgACACCggCTCCTggTATATgA-3'. The PCR reaction was performed in 100  $\mu$ L volumes containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ L of each dNTP, 50 ng of genomic DNA, 100 pmol of each primers and 2.5 units of *Taq* DNA polymerase. Amplification was as follows: initial denaturation at 95°C for 5 min, annealing at 64°C for 1 min, extension at 74°C for 3 min and final extension of 10 min in a DNA Thermal Cycler (Perkin- Elmer Cetus Corp). After DNA amplification, the PCR products were digested with *Hae*III and restriction fragments were then separated by electrophoresis in 10 % polyacrylamide gels. The gel was stained with ethidium bromide and visualized under UV light.

### Statistical Analysis

The following linear model was used to test the effects of the  $\beta$ -LG variants on first parity production of milk, fat and protein yield and on fat and protein percentage. The PROC GLM procedure of the SAS (SAS, Inst. Inc., Cary NC) was used for statistical analyses.

$$Y_{ijkl} = \mu + H_i + (Y \times S)_j + \beta\text{-LG}_k + e_{ijkl}$$

$Y_{ijkl}$  = the production record of the  $l^{\text{th}}$  cow

$\mu$  = overall means

$H_i$  = the effect of the  $i^{\text{th}}$  herd

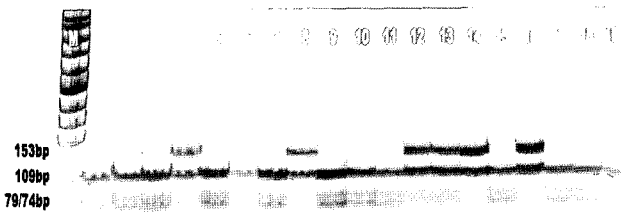
$(Y \times S)_j$  = the effect of the  $j^{\text{th}}$  year and season of calving

$\beta\text{-LG}_k$  = the effect of the  $k^{\text{th}}$   $\beta$ -LG variant ( $\beta$  =AA, AB or BB)

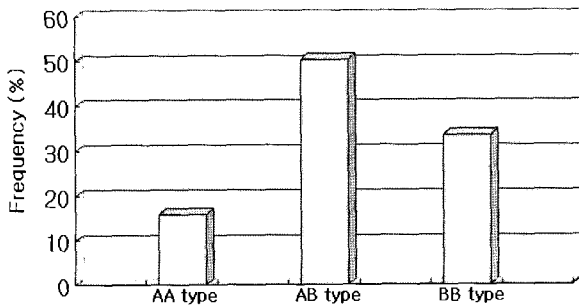
$e_{ijk}$  = the random residual associated with each record.

**RESULTS**

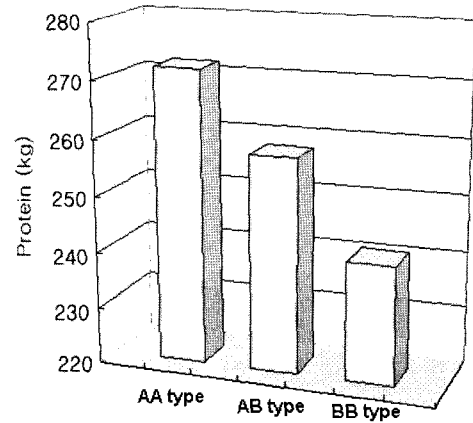
We have amplified an 262 bp fragment between nucleotides 367 and 629 from exon IV and intron IV of the bovine  $\beta$ -LG gene. Fig. 1 shows the restriction patterns of  $\beta$ -LG variants after digestion with the *Hae*III enzyme. Digests of variant A products yielded two fragments of 109 and 153 bp, digests of variant B products resulted in three fragments of 109, 79 and 74 bp and the AB heterozygous variant showed all these DNA bands. Three different  $\beta$ -LG variants AA, AB and BB were observed, with frequencies of 15.9, 50.5 and 33.6 %, respectively (Fig. 2). Estimated allelic frequencies were 0.412 and 0.588 for A and B. The results from the analysis of effects of  $\beta$ -LG variants on milk yield and composition are shown in



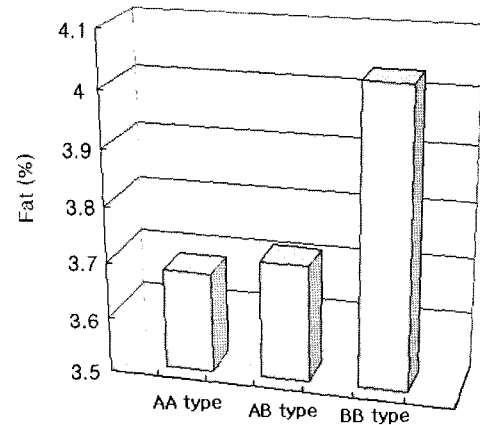
**Fig. 1.** PCR-RFLP patterns of  $\beta$ -LG variants in 10% polyacrylamide gel following digestion with *Hae*III enzyme of PCR products. In the gel, lanes 14 and 16 represent AA type, lanes 1, 2, 3, 5, 6, 7, 9, 10, 11, 15, 17, 18 and 19 represent BB type and lanes 4, 8, 12 and 13 represent AB type. M represents the molecular size marker ( $\Phi$ X-174 DNA/*Hae*III marker).



**Fig. 2.** Frequencies of  $\beta$ -LG variants AA, AB and BB in domestic dairy cow population.



**Fig. 3.** Association between  $\beta$ -LG variants and protein yield for first lactation in Holstein dairy cattle.



**Fig. 4.** Association between  $\beta$ -LG variants and fat percentage for first lactation in Holstein dairy cattle.

Table 2. The  $\beta$ -LG variant affected protein yield ( $p < 0.05$ ) and fat percentage ( $p < 0.05$ ). Cows with the AA variant produced higher protein yield than cows with BB variant ( $p < 0.05$ ). AA type cows produced 31kg of milk protein more than BB type cows (Fig. 3). On the other hand, cows with BB variant had higher fat percentage compared with AA or AB variant cows ( $p < 0.05$ ). BB type cows produced more fat by 0.35 and 0.32 percentage, respectively, compared with AA and AB type cows (Fig. 4). However, milk yield, fat yield and protein percentage were not significantly affected by the  $\beta$ -LG variant.

**DISCUSSION**

The  $\beta$ -LG, a molecule of 162 amino acids, is a major component of whey protein and has seven known protein variants (A, B, C, D, E, F and G) in various cattle breeds

Table 2. Least square means and standard errors of  $\beta$ -LG variant for the milk production traits

$\beta$ -LG type	Milk production traits				
	Milk	Fat	Protein	Fat	Protein
	(Kg)			(% )	
AA	8,014±233.7	298±14.62	272±16.28 <sup>a</sup>	3.68±0.16 <sup>a</sup>	3.16±0.15
AB	7,916±272.5	292±17.35	258±14.71 <sup>a</sup>	3.71±0.18 <sup>a</sup>	3.14±0.14
BB	7,835±254.6	304±15.27	241±15.43 <sup>b</sup>	4.03±0.14 <sup>b</sup>	3.12±0.16
P	-	-	*	*	-

\*  $p < 0.05$ .<sup>a,b</sup> Means with different superscripts in the same column are significantly different ( $p < 0.05$ ).

(Eigel *et al.*, 1984). In Holstein dairy cattle, only two common genetic variants,  $\beta$ -LG A and B, were found.  $\beta$ -LG variants exhibit differences in their physiochemical behavior on both native milk and model systems. The structure of  $\beta$ -LG was recently reported to consist of antiparallel  $\beta$ -sheets formed by nine  $\beta$ -strands,  $\alpha$ -helix and turns, and amino acid residues in a random nonrepetitive arrangement without a well-defined structure (Imafidon and Ng-Kwai-Hang, 1991).  $\beta$ -LG variants A and B differ in single amino acid substitutions at positions 64 and 118 (Eigel *et al.*, 1984). At position 64, Asp (GAT) is changed to Gly (GGT) and at position 118, Val (GTC) is changed to Ala (GCC), for A and B variants, respectively (Jamieson *et al.*, 1987). These differences in their primary sequence could modify their conformational changes and interactions with other milk constituents. The technological properties of milk are greatly dependent on the amount and proportion of each milk constitution. The milk proteins, because of their variability in concentrations and types, contribute significantly in this aspect. Bovine  $\beta$ -LG cDNA has been cloned and sequenced (Alexander *et al.*, 1989; Jamieson *et al.*, 1987). At the DNA level, these changes arise from two single base substitutions, A to G and a T to C, respectively. The change at position 118 creates a *Hae*III site (GG/CC) in  $\beta$ -LG B variant, but not in  $\beta$ -LG A variant (Lien *et al.*, 1990). This makes it possible to discriminate between  $\beta$ -LG A and B variants directly at the DNA level. Therefore, this PCR-RFLP method followed by *Hae*III digest allows discrimination between  $\beta$ -LG genotypes AA, AB and BB at the molecular level.

In the  $\beta$ -LG system, all three genotypes were present with frequencies ranging from 0.34 to 0.16. These frequencies were similar to those reported in the literature for Holstein popu-

lations (Aleandri *et al.*, 1990; Famula and Medrano, 1994; Ng-Kwai-Hang *et al.*, 1986). The gene frequencies of variants A and B are similar to those of previous reports (Chung *et al.*, 1996; Chung *et al.*, 1998) in domestic Holstein cow population. In the association test between  $\beta$ -LG system and milk production traits, increased protein yield associated with the  $\beta$ -LG AA type was found in this study. The heterozygote AB genotype was intermediate, implying an additive locus for this trait. This result is consistent with reports by Aleandri *et al.* (1990), Bovenhuis *et al.* (1992) and Ng-Kwai-Hang and Krockner (1984). The AA genotype of  $\beta$ -LG has been shown to have a favorable effect on protein yield. Therefore, to improve the milk protein yield it may be proposed to increase the frequency of  $\beta$ -LG AA type. However, Lunden (1997) suggested a positive effect of the  $\beta$ -LG B variant on casein content and on the ratio to total protein. From the results by Schaar *et al.* (1985), the relationship between casein number and cheese yield can be derived. Based on those calculations, the casein number in milk from  $\beta$ -LG BB cows was 3% higher than that from  $\beta$ -LG AA cows; this difference corresponds to a 2% increase in cheese yield.

Fat percentage was significantly associated with the genotype of  $\beta$ -LG (Aleandri *et al.*, 1990; Bovenhuis *et al.*, 1992; Hill, 1993; McLean *et al.*, 1984; Ng-Kwai-Hang *et al.*, 1984, 1986). The B variant was favorable, with BB homozygotes producing significantly more than AA type. The superiority of the  $\beta$ -LG B variant for fat content found in this study is in agreement with the findings of Bovenhuis *et al.* (1992), Hill (1993), McLean *et al.* (1984) and Ng-Kwai-Hang *et al.* (1984, 1986). Although genotypes at the  $\beta$ -LG system had no significant effect on first lactation yield, the AA type had a higher average milk yield than the BB types. Recently, Tsiaras *et al.*

(2005) reported that cows with AB and BB genotypes for  $\beta$ -LG produced more fat yield by 27 and 33 kg, respectively, compared with  $\beta$ -LG AA cows, suggesting possible dominance of the B variant. Other researchers, however, have shown no significant association between fat yield and  $\beta$ -LG genotypes (Bovenhuis *et al.*, 1992; Lunden *et al.*, 1997; Ojala *et al.*, 1997; Van Eenennaam and Medrano, 1991).

In studies of  $\beta$ -LG genotypic effects on milk yield, several authors have reported no significant associations (Lunden *et al.*, 1997; Ojala *et al.*, 1997; Van Eenennaam and Medrano, 1991). However, reports exist where  $\beta$ -LG genotype AA (Aleandri *et al.*, 1990; Bovenhuis *et al.*, 1992) have been positively associated with milk yield. Consequently, the our results indicated that the  $\beta$ -LG A variant is associated with higher milk yield and protein yield, and the B variant appears to be associated with higher fat % in milk. Therefore, milk production traits could be improved through  $\beta$ -LG typing by increasing the frequency of A variant for protein yield or the frequency of B variant for fat content in Holstein dairy cattle populations. In particular, rapid progress could be achieved by selecting sires bearing the favorable  $\beta$ -LG variant.

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