

## Association of Candidate Genes with Production Traits in Korean Dairy Proven and Young Bulls

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**ABSTRACT :** This study was performed to offer effective basic data for selection and improvement of Korean dairy cattle through identifying distributional properties among candidate genes (bovine butyrophilin, signal transducers and activators of transcription 5a, and prolactin hormone). In this study, polymorphisms of candidate genes were identified and the relationships between loci and production traits of each gene were analyzed using frozen semen of Holstein bulls (19 proven and 77 candidates). In butyrophilin (BTN) locus, polymorphisms information contents (PIC) value of BTN2 (0.372) was higher than those of others (BTN1; 0.155, BTN3; 0.254, BTN4; 0.169). As a result of analysis of genotyping STAT5a, using single stranded conformational polymorphism (SSCP) method and microsatellite locus, PIC values were 0.189 and 0.457, respectively. And PIC value of prolactin hormone gene was 0.176. In the relationships between genotypes and production traits, BTN3 was associated with 305-day production traits ( $p < 0.05$ ). PTAs for B allele were such as 110.43, 88.28 and 75.25 in BTN1, 3, 4 and these values were higher than those of A allele, but in the case of BTN2, A allele with 154.19 was higher than that of B allele. The results obtained from using candidate genes may be used as an useful index for the genetic improvement of dairy cattle population in Korea, and further studies are needed. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 2 : 165-169)

**Key Words :** Butyrophilin, Signal Transducers and Activators of Transcription 5a, Prolactin Hormone, Polymorphisms Information Contents, 305-day Production Traits, Predicted Transmitting Ability

### INTRODUCTION

Butyrophilin (BTN) is the major integral protein that is associated with the milk fat globule membrane (MFGM) of many species, and comprises 40% of the total protein composition of the MFGM by weight in Holstein, and 20% of the total protein composition of the MFGM by weight in Jersey (Stocklin et al., 1996). BTN gene locates on the bovine chromosome 23, and specifically expressed on the apical surface of mammary epithelial cells during lactation and becomes incorporated as an integral protein into the MFGM during the budding and secretion of fat droplets into milk (Franke et al., 1981). So many results were reported that expression increased during the last half of pregnancy and was maximal during lactation (Mather et al., 1993; Ogg et al., 1996; Banghart et al., 1998; Davey et al., 1999). BTN is a member of the Immunoglobulin Superfamily (IgSF; Gardinier et al., 1992), and Taylor et al. (1996) reported that the chromosomal location of BTN within the MHC of man and cattle and its sequence homology to members of the IgSF may suggest a possible immunologic function.

Signal Transducers and Activators of Transcription 5a (STAT5a) known as Mammary Gland Factor (MGF), is mediators of prolactin signals in target tissues, including the mammary gland (F. Gouilleux, 1994). The role of STAT5a

in mammary glands was first analysed in STAT5a knock-out mice. Possible functions by gene-targeted mice are mammary gland development, lobulo-alveolar mammapoiesis, lactation, activation of milk protein genes, and partial inhibition of T-cell growth (Takeda and Akira, 2000). And it is known as regulatory factor for the promoter of  $\beta$ -casein gene (Liu, 1997). Recently, Lee et al. (2002) reported polymorphisms of BTN and STAT5a gene in Korean dairy cattle.

This study was performed to offer effective basic data for selection and improvement of Korean dairy cattle through analyzing genotypes and identifying distributional properties among candidate genes. And this study was also carried out to examine the possibility for discovering QTLs with candidate genes and for development of marker assist selection.

### MATERIALS AND METHODS

#### Materials

This study was performed using 19 Holstein proven and 77 candidate bulls' (total 96 Holstein bulls) frozen semen from Dairy Cattle Improvement Center, National Agricultural Cooperation Federation.

#### Method

*DNA extraction from semen :* Genomic DNA was prepared from frozen semen by the modified methods of Lee et al. (1995a,b) and diluted in 3<sup>rd</sup> distilled water to 25 ng/ $\mu$ l.

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**Table 1.** Oligonucleotide sequences of each loci specific DNA marker

Loci	Primer sequence	Position of chromosome	Gene bank accession No.
BTN1	5'-TGG AGC TCT ATG GAA ATG GG-3' 5'-CTA CCC AAC AGG AAG AAA CAG-3'	23 (exon7)	AF005497
BTN2	5'-GAT CCC TCA TGC CTG GAA TAT G-3' 5'-GTT GCC CTT GAC CTT TAG TGG A-3'	23 (intron2)	AF005497
BTN3	5'-CTG AAG TTC CCG ACA AAC TCG-3' 5'-CTC TGC ATC TTC ACC CAC CAC-3'	23 (exon2 ~ exon3)	Z93323
BTN4	5'-CTT CTT CCC AAG GCT GAC-3' 5'-CTT ACT GAG CTC TTC CAG G-3'	23 (exon5 ~ exon6)	Z93323
STAT5a	5'-CTT GGG AGA ACC TAA CAT CAC T-3' 5'-AGA CCT CAT CCT TGG GCC-3'	19 (intron15 ~ exon 6; SH2)	AF079568
STAT5a MS	5'-TCT CCT TTC CTG GAT CTT TCT CAC-3' 5'-GGG AGA GAA GAA AAG GGA AAA GAT T-3'	(TG)n	U96644
PRL	5'-CGA CTC CTT ATG AGC TTG ATT CTT-3' 5'-GCC TTC CAG AAG TCG TTT GTT TTC-3'	23 (exon3)	V00112

BTN: Butyrophilin, STAT5a: Signal Transducers and Activators of Transcription 5a, STAT5a MS: STAT5a microsatellite, PRL: Prolactin.

**Table 2.** PCR annealing temperature, expected fragment size, and used endonucleases of each locus

Loci	Annealing temp. (°C)	Expected fragment size (bp)	Restriction enzyme
BTN1	60	501	<i>Hae</i> III (AAG → AGG)
BTN2	52	568	<i>Alu</i> I (AGCT → ATCT)
BTN3	61	576	<i>Taq</i> I (ACAA → TCGA)
BTN4	59	683	<i>Mbo</i> I (GATC → AATC)
STAT5a	60	379	-
STAT5a MS	63	114	-
PRL	63	156	<i>Rsa</i> I (GTG → GTA)

**Primer synthesis** : After identifying the information about DNA sequences of each locus provided by Gene Bank (<http://www.ncbi.nlm.nih.gov>), selected and designed optimal primers for PCR, and primer sequences of each locus were shown in Table 1.

**Polymerase chain reaction** : PCR amplification (final volume : 25 µl) was performed on 50 ng genomic DNA in 0.25 mM of each dNTP, 10 pmole each primer, and 1 U of Taq DNA polymerase using an MJ research thermocycler (PTC 200, USA).

**Enzyme digestion** : The four BTN and PRL PCR products were digested with *Hae* III (BTN1), *Alu* I (BTN2), *Taq* I (BTN3), *Mbo* I (BTN4), and *Rsa* I (PRL), respectively. All enzymes were reacted 10 Unit in 37°C water bath.

**Electrophoresis for genotype analysis** : The enzyme digested fragments were separated in 3% Nusieve 3:1 agarose gel. The amplified PCR products of STAT5a microsatellite locus analyzed using ABI 310 Genetic Analyser (Perlin-Elmer Co., USA).

**Single stranded conformational polymorphism (SSCP)** : Electrophoresis was performed for 5 h at 13 W in denatured polyacrylamide gel. The DNA bands were visualized by silver staining method.

### Statistical analysis

**Polymorphisms Information Content (PIC)** : Expected PIC value of each locus was calculated by using the method of Botstein et al. (1980).

$$PIC = 1 - \sum_{i=1}^n P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2P_i^2 P_j^2$$

with,  $p_i$  = frequency of the  $i$ th allele  
 $p_j$  = frequency of the  $j$ th (=  $I+1$ ) allele  
 $n$  = number of alleles

**Association test between genotype and production traits** : Variance component was estimated using by animal model and derivative-free REML. PTA value was calculated a half of estimated breeding value (EBV). Association test was carried out using 92 bulls that have records of progeny test in total 96 bulls, and the model used is as follows;

$$Y = \mu + HYS + G + E$$

With, Y = observations of production traits

$\mu$  = overall average

HYS = effects of Herd-Year-Seasons

G = genotype effects of each individuals

E = random residual errors

## RESULTS

### Identification of genetic polymorphisms

**Butyrophilin** : As a result of genotyping BTN, we identified AA, AB and BB types in BTN2, but only AA and AB types were identified in others. PIC value of BTN2

**Table 3.** Genotypes, gene frequencies, and PIC values of each BTN locus in proven and candidate young bulls

Loci	Genotype	Gene frequency	PIC value
BTN1	AA	A=0.906	0.155
	AB	B=0.094	
BTN2	AA	A=0.552	0.372
	AB	B=0.448	
	BB		
BTN3	AA	A=0.818	0.254
	AB	B=0.182	
BTN4	AA	A=0.896	0.169
	AB	B=0.104	
Total (96*)		1.000	

\* Total individual number.

**Table 4.** Genotypes, gene frequencies, and PIC values of STAT5a locus in proven and candidate young bulls

Loci	Genotype	Gene frequency	PIC value
STAT5a	AA	A=0.880	0.189
	AB	B=0.120	
	BB		
STAT5a MS**	110/110	110=0.245	0.457
	110/112	112=0.641	
	110/114	114=0.115	
	112/112		
	112/114		
	114/114		
Total (96*)		1.000	

\* Total individual number. \*\* Microsatellite locus.

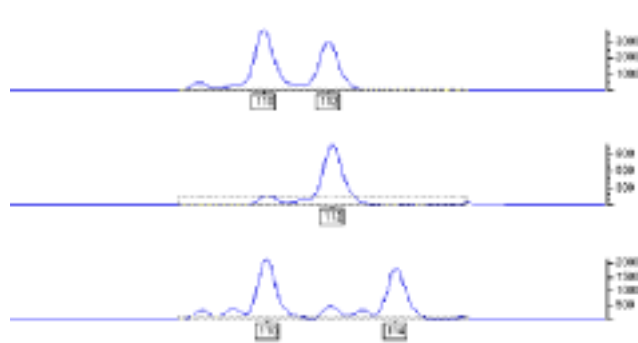
(0.372) was higher than others (BTN1; 0.155, 3; 0.254 and 4; 0.169). These values were similar to previous results (BTN1; 0.1664, 2; 0.3724, 3; 0.2549 and 4; 0.1794) by Lee et al. (2002). Table 3 shows the gene frequencies and PIC values of each BTN locus.

**STAT5a** : As a result of analysis of genotyping STAT5a using SSCP, AA, AB and BB types were identified, and BB type was found only in two individuals among 96 bulls, and PIC value was 0.189. The polymorphisms of STAT5a microsatellite locus that analyzed by using ABI 310 Genetic Analyser (Perlin-Elmer Co., USA) were detected (Figure 1), TC repeats 10 (110 bp), 11 (112 bp) and 12 (114 bp) were found, allele frequencies for 110 bp, 112 bp, 114 bp were 0.245, 0.641 and 0.115, respectively, and PIC value was 0.457 (Table 4).

**Prolactin** : In prolactin hormone gene for 96 bulls, the gene frequencies of A, B were 0.891 and 0.109, respectively, and PIC value was 0.176 (Table 5).

#### Association between candidate gene's genotypes with production traits

**305-day production traits** : As a result of examining the relationships test between genotypes and 305-day production traits, BTN3 was associated with milk yield, fat yield, protein yield, and SNF yield. Averages (7,316.45, 271.33, 234.73 and 641.24) of AB type in BTN3 were

**Figure 1.** Electropherogram of STAT5a fragment (Fam), run on ABI prism 310 genetic analyzer.**Table 5.** Genotypes, gene frequencies and PIC values of prolactin locus in proven and candidate young bulls

Locus	Genotype	Gene frequency	PIC value
Prolactin	AA	A=0.891	0.176
	AB	B=0.109	
Total (96*)		1.000	

\* Total individual number.

higher than those (6,961.98, 258.00, 222.95 and 605.44) of AA type, but STAT5a and prolactin gene were not significantly different.

**Predicted transmitting ability (PTA)** : After obtaining the predicted transmitting abilities, genotypes effects of B allele being such as 110.43, 88.28 and 75.25 in BTN1, 3, and 4 were higher than those of A allele, but in the case of BTN2, A allele with 154.19 was higher than this of B allele. And 114 bp allele (12 repeated TG sequences) in STAT5a microsatellite was higher than others (Table 7).

## DISCUSSION

Candidate genes for given traits are sequenced genes of known biological roles that are involved with the developmental or physiological roles of the specific trait. These genes may be structural genes or regulatory genes or genes affecting the biochemical pathway of the expression.

In order to prepare the basic foundation for studying candidate genes, we have to do, firstly, choosing the candidate genes, designing primer sequences to amplify the gene, uncovering polymorphisms in the gene, and developing a convenient procedure for genotyping the polymorphic sites. The second step is about studying candidate gene itself, identifying a suitable population, carrying out the association test, and verifying the results (Rothschild and Soller, 1997).

In this study, we performed two steps, firstly, we designed oligonucleotide primer pairs of BTN1, 2, 3, 4 and STAT5a SSCP, STAT5a microsatellite locus, and also prolactin gene with a view to detect the polymorphic region. And we examined the relationship between polymorphic regions with production traits data in Korean proven and

**Table 6.** 305-day production traits (Mean  $\pm$  STD) of each genotype and probability of each BTN locus in proven and candidate young bulls

Genotype	BTN1		Prob.	BTN2			Prob.
	AA	AB		AA	AB	BB	
Milk305	7,038.92 $\pm$ 661.15	7,295.51 $\pm$ 597.19	0.1363	7,323.51 $\pm$ 645.68	7,062.98 $\pm$ 670.93	6,807.99 $\pm$ 445.64	0.0945
FAT305	261.40 $\pm$ 29.41	268.48 $\pm$ 29.46	0.3620	268.13 $\pm$ 29.29	263.27 $\pm$ 30.47	250.36 $\pm$ 20.91	0.2697
PROT305	225.78 $\pm$ 21.37	232.92 $\pm$ 18.78	0.1971	235.30 $\pm$ 23.38	225.94 $\pm$ 20.77	219.29 $\pm$ 13.13	0.0915
SNF305	613.95 $\pm$ 57.53	636.12 $\pm$ 48.55	0.1351	635.35 $\pm$ 57.10	616.22 $\pm$ 58.46	598.74 $\pm$ 33.84	0.1990

Genotype	BTN3		Prob.	BTN4		Prob.
	AA	AB		AA	AB	
Milk305	6,961.98 $\pm$ 610.76	7,316.45 $\pm$ 676.18	0.0119	7,050.40 $\pm$ 658.98	7,237.92 $\pm$ 629.36	0.2679
FAT305	258.00 $\pm$ 27.09	271.33 $\pm$ 31.80	0.0363	261.65 $\pm$ 29.32	267.14 $\pm$ 30.10	0.4713
PROT305	222.95 $\pm$ 17.67	234.73 $\pm$ 24.41	0.0091	225.91 $\pm$ 21.54	232.04 $\pm$ 18.43	0.2591
SNF305	605.44 $\pm$ 49.15	641.24 $\pm$ 61.61	0.0030	614.67 $\pm$ 57.20	632.20 $\pm$ 51.92	0.2288

BTN: butyrophilin, Milk305: 305-day milk yield, FAT305: 305-day fat yield.

PROT305: 305-day protein yield, SNF305: 305-day SNF yield, Prob.: probability.

**Table 7.** Genotypic differences of BTN, STAT5a, and PRL for production traits

Genotype	BTN1		BTN2			BTN3	
	AA	AB	AA	AB	BB	AA	AB
PTA Milk	32.30	110.43	154.19	23.56	-12.96	24.83	88.28
PTA FAT	0.36	1.07	2.07	0.27	-1.10	0.21	1.02
PTA PROT	1.93	4.76	5.40	1.77	1.15	1.72	3.83
PTA SNF	5.16	11.38	12.91	4.97	2.33	3.81	10.97

Genotype	BTN4		PRL		STAT5a	
	AA	AB	AA	AB	AA	AB
PTA Milk	40.39	75.25	47.90	46.51	30.39	110.71
PTA FAT	0.25	1.47	0.50	0.48	-0.40	3.81
PTA PROT	2.26	3.33	2.18	3.50	2.25	3.26
PTA SNF	5.84	8.46	6.40	6.30	5.12	11.04

Genotype	STAT5a microsatellite					
	110/110	110/112	110/114	112/112	112/114	114/114
PTA Milk	-25.59	78.80	116.55	17.86	59.19	228.10
PTA FAT	-3.51	3.03	3.78	-0.99	1.09	3.00
PTA PROT	-0.61	2.51	5.25	2.29	2.95	7.70
PTA SNF	-3.34	8.23	23.58	3.81	9.50	7.95

PTA: predicted transmitting ability, BTN: butyrophilin, PRL: prolactin, STAT5a: signal transducers and activators of transcription 5a.

young bulls.

Although Davey et al. (1997) reported the structure of BTN gene and Seyfert and Leuthen (1998) reported that the genes were different in terms of the number of exons, and they showed a near 100% similarity to nucleotide sequences and the same expression pattern.

The PIC value of BTN2 (0.373) was highest among BTN loci (BTN1; 0.170, BTN3; 0.298, BTN4; 0.188). The present value was relatively higher than previous results that reported kappa-casein (0.2150) and beta-lactoglobulin (0.3544) (Chang et al., 2001), therefore it could be useful for a genetic marker due to its higher value. In addition, the present study showed that relatively low PIC value (0.189) was detected by analyzing STAT5a with SSCP technique. In microsatellite locus, 110, 112 and 114 alleles were detected with frequencies of 0.245, 0.641 and 0.115, respectively. These results were not in accordance with the observation of McCracken et al. (1997) who reported that frequencies of

alleles were 0.13, 0.82 and 0.05, respectively. Genotypic frequencies such as AA and AB types were 0.7812 and 0.2188, respectively, in prolactin loci. Chung et al. (1996) also reported the similar results that AA and AB type frequencies of prolactin loci were 0.621 and 0.218, respectively. However, gene frequencies of A and B were different from observations of chung et al. (1996) and those of present study (0.891 and 0.109 vs. 0.730 and 0.270, respectively).

BTN3 was associated with 305-day production traits (milk and fat yield,  $p < 0.05$ ; protein and SNF yield,  $p < 0.01$ ). PTA values of production traits (milk, fat, protein and SNF yield) of B allele in BTN1, 3 and 4 were higher than those of A allele however, in the case of BTN2, A allele was higher than B allele. PTA values of B allele and 114 bp allele in STAT5a gene were higher, and prolactin gene was not associated with 305-day production traits and PTA values of production traits (Tables 6 and 7).

In this study, butyrophilin gene could be an important marker for dairy cattle breeding, considering the fact that the gene was not only associated with production traits, but also with disease traits. Then further studies about functions of BTN and STAT5a gene in dairy cattle must be performed, and then BTN and STAT5a gene will be useful genetic marker for MAS. Therefore, the wide and various researches should be performed in candidate genes such as butyrophilin and STAT5a for the genetic improvement of dairy cattle, and be proposed as an useful index for selection improvement.

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