Genetic Polymorphisms of BTN and STAT5a Genes in Korean Proven and Young Bulls

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ABSTRACT : This study was performed to detect polymorphisms of the two candidate genes, bovine BTN (Butyrophilin) and STAT5a (Signal Transducers and Activators of Transcription) gene using 98 Holstein bulls' frozen semen, and to offer the basic information for QTL (Quantitative Trait Loci) analysis. Each BTN PCR product was digested with endonuclease restriction enzyme. The digested fragments of four BTN PCR products were observed as follows: 316, 280, and 162 bp in BTN1, 568, 305 and 263 bp in BTN2, 576, 332, and 244 bp in BTN3, and 573, 291, and 282 bp in BTN4, respectively. The gene frequencies of A and B allele in four BTN loci were as follows: 0.8980 and 0.1020 in BTN1, 0.5510 and 0.4490 in BTN2, 0.8163 and 0.1837 in BTN3, and 0.8875 and 0.1122 in BTN4, respectively. And three genotypes (homotype1, heterotype, and homotype2) for STAT5a were observed by SSCP (single stranded conformational polymorphism) method and the genotype frequencies are 78.57%, 19.39%, and 2.04%, respectively. The PIC (Polymorphism Information Content) value and heterozygosity of four BTN loci were as follows: 0.1695 and 0.1870 in BTN1, 0.3713 and 0.4927 in BTN2, 0.2549 and 0.2999 in BTN3, and 0.1794 and 0.1992 in BTN4, respectively. Comparing with the reported data, PIC value of BTN2 might have the possibility to be useful marker. Other BTN loci indicated skewed allele distribution. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 938-943)***

Key Words : Butyrophilin, Signal Transducers and Activators of Transcription, Quantitative Trait Loci, Polymorphism Information Content, Heterozygosity

INTRODUCTION

Many studies reported milk protein genes (casein and whey proteins) as candidate genes (Lee et al., 1995, 1996; Ng-Kwai-Hang, 1998). However, a few studies have been reported in other loci except for casein and whey proteins for candidate gene study. In this study, therefore, we chose Butyrophilin (BTN) and Signal Transducers and Activators of Transcription 5a (STAT5a) as a candidate gene and then performed candidate gene study, because bovine BTN and STAT5a proteins have an important role in biological and physiological system of dairy cattle.

Butyrophilin is a glycoprotein that is secreted in association with the milk fat globule membrane from mammary epithelial cell (Ogg et al., 1996). It has been estimated to comprise 34 to 43% of the total milk fat globule membrane protein in Holstein milk and approximately 20% in Jersey milk (Mather et al., 1993; Mather, 2000). Since it is associated with genes in the major histocompatibility complex (MHC) (Vernet et al., 1993; Ashwell et al., 1996; Taylor et al., 1996) and it is specifically expressed in the mammary gland during late pregnancy and lactation (Mather and Jack, 1993), which has attracted attention recently.

Signal Transducers and Activators of Transcription 5a and 5b are discretely encoded transcription factors that mediate signals for a broad spectrum of cytokines. STAT protein family, consisted of seven members (STAT1, 2, 3, 4, 5a, 5b, and 6), is involved in cytokine signal transduction (Takeda and Akira, 2000). 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 mammopoiesis, lactation, activation of milk protein genes, and partial inhibition of T-cell growth (Takeda and Akira, 2000).

Since the BTN and STAT5a genes have a significant role of in this pathway, these genes may be candidate genes for dairy cattle quantitative traits (Taylor et al., 1996; Antoniou et al., 1999). The goal of this study is to discover the polymorphisms and develope a convenient genotyping procedure and characterize the distributional properties of BTN and STAT5a genes in Korean dairy cattle (Holstein proven and young bulls).

MATERIALS AND METHODS

Materials

This study was performed using 98 Holstein bulls' frozen semen from Dairy Cattle Improvement Center, National Agricultural Cooperation Federation.

Methods

DNA extraction from semen : Genomic DNA was prepared from frozen semen by the method of Lee et al.

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(1995) and diluted in distilled water to 25 ng/μl. Then, the diluted DNA was used for template DNA for Polymerase Chain Reaction (PCR).

Primer synthesis : Each primer was designed according to the published bovine BTN sequence and bovine STAT5a sequence available from Genebank.

Polymerase chain reaction : PCR amplification (50 μl final volume) was performed on 100 ng of genomic DNA in 0.25 mM of each dNTP, 0.4 μM of forward and reverse primer (0.6 μM in Stat5a), and 1 U of Taq DNA polymerase using an MJ research thermocycler (PTC100, USA).

Enzyme digestion of four BTN loci : The four BTN PCR products were digested with HaeIII(BTN1), Alu I (BTN2), Taq I (BTN3), and Mbo I (BTN4), respectively. In enzyme digestion, 10 Unit of restriction enzyme was used and the reaction was performed at 37°C in 5 h to overnight.

Electrophoresis for genotype analysis : The amplified PCR products were confirmed in 2% ethidium-bromide stained agarose gel. And the digested fragments of four BTN loci were separated in 3% agarose gel.

Single stranded conformational polymorphism (SSCP) and silver staining of STAT5a : An SSCP gel-loading buffer was added to the PCR product and the mixture was vortexed for a few seconds. The samples were denatured at 95°C for 5 min and cooled to 4°C. Electrophoresis was performed for 5 h at 13 W. The DNA bands were visualized by silver staining method.

Statistical analysis

Expected Polymorphism Information Content (PIC) value of each BTN locus was calculated by using the method of Botstein et al. (1980) and expected heterozygosity of each BTN locus was calculated by using the method of Hubert et al. (1992).

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

with, p_i = frequency of the ith allele,

p_j = frequency of the jth (=I+1) allele,

n = number of alleles

$$Heterozygosity = 1 - \sum_{i=1}^{n} P_i^2$$

with, p_i = frequency of the ith allele

RESULTS

PCR amplification

Four BTN PCR products and a Stat5a PCR product were loaded in 2% agarose gel and visualized by EtBR staining. The bands of four BTN PCR products were 501, 568, 576, and 683 bp, respectively. The band of Stat5a PCR product was 379 bp. Figure 1 shows the bands of PCR products and their relative base pair.

Enzyme digestion of four BTN loci PCR products

Based upon the sequence of Jack and Mather (1990), digestion with HaeIII of BTN1 PCR product yielded 316, 162, 13, and 10 bp fragments in AA type. However, in AB type, an additional band of 280 bp was observed. BTN2 PCR product was digested with Alu I producing two fragments of 305 and 263 bp (A-allele). However, B-variant was not digested with Alu I restriction enzyme. According to the sequence of the Zegeye et al. (1999), BTN 3 and 4 were digested with Taq I and Mbo I endonuclease, respectively. Digestion with Taq I of BTN3 PCR product yielded 332 and 244 bp fragments in B-allele. Therefore, in AB type, three fragments of 576, 332, and 244 bp were observed. In BTN4, digestion with Mbo I of PCR product yielded three fragments of 291, 282, and 110 bp in A-allele. However, one of two restriction site within B-allele was not digested due to the point mutation. Thus, only two fragments of 573 and 110 bp were observed. Figure 2 shows the each fragment of digested PCR products.

Table 2 shows the genotype frequency and fragment size of each BTN locus and table 5 shows the PIC value and heterozygosity of each BTN locus. In BTN2 locus, BB-type which was not observed in other three BTN loci was

Table 1. Oligonucleotide sequences of each locus specific DNA primer

Loci	Primer sequence	Gene bank accession number	Reference
BTN1	5'-TGG AGC TCT ATG GAA ATG GG-3' 5'-CTA CCC AAC AGG AAG AAA CAG-3'	AF005497	Taylor et al., 1996
BTN2	5'-GAT CCC TCA TGC CTG GAA TAT G-3' 5'-GTT GCC CTT GAC CTT TAG TGG A-3'	AF005497	Husaini et al., 1999
BTN3	5'-CTG AAG TTC CCG ACA AAC TCG-3' 5'-CTC TGC ATC TTC ACC CAC CAC-3'	Z93323	Zegeye et al., 1999
BTN4	5'-CTT CTT CCC AAG GCT GAC-3' 5'-CTT ACT GAG CTC TTC CAG G-3'	Z93323	Zegeye et al., 1999
STAT5a	5'-CTT GGG AGA ACC TAA CAT CAC T-3' 5'-AGA CCT CAT CCT TGG GCC-3'	AF79568	Antoniou et al., 1999

BTN: Butyrophilin, STAT5a: Signal transducers and activators of transcription 5a

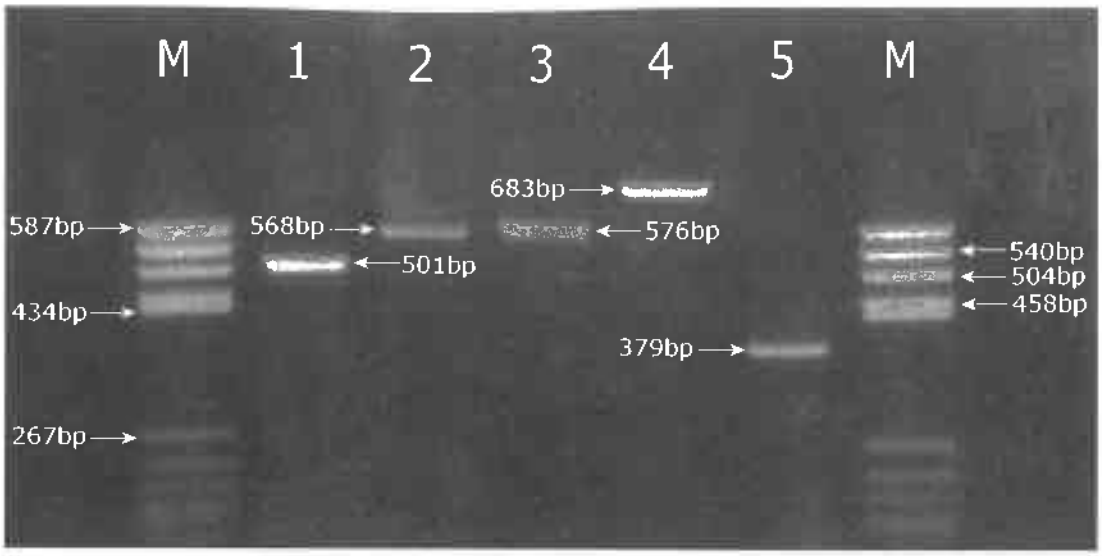

Figure 1. The PCR products and their relative base pair (M: pBR322 HaeIII digested marker, Lane 1: BTN1 PCR product, Lane 2: BTN2, Lane 3: BTN3, Lane 4: BTN 4, and Lane 5: STAT5a PCR product).

Figure 2. Restriction fragments length polymorphism of four BTN loci (M: pBR322 HaeIII digested marker, Lane 1: AA type of BTN 1, Lane 2: AB, Lane 3: AA type of BTN 2, Lane 4: AB, Lane 5: BB, Lane 6: AA type of BTN 3, Lane 7: AB, Lane 8: AA type of BTN 4, Lane 9: AB).

observed and more B-variants were observed than any other loci. In BTN3, homozygous type of B-allele was not observed, but as compared with BTN1 and 4, more B-variants were observed than other two BTN loci (BTN1 and BTN4). However, in BTN1 and BTN4 loci, BB type was not observed and the gene frequency of B-variant was very low.

Taylor et al. (1996) reported the estimated gene frequencies of BTN1 as 0.875 and 0.125 for alleles A and B, respectively. Also Husani et al. (1999) reported those of BTN1 as 0.85 and 0.15, and those of BTN2 as 0.56 and 0.44 for alleles A and B, respectively. Comparing with these data reported, this study showed similar result. The gene frequencies of BTN1 were 0.8980 and 0.1020, and those of BTN2 were 0.5510 and 0.4490, respectively. On the other hand, the gene frequencies of BTN3 and BTN4 have not been reported. In this study, the gene frequencies of BTN3 were observed as 0.8163 and 0.1837 for alleles A and B, and those of BTN4 as 0.8875 and 0.1122, respectively. And we could not find BB type in BTN3 and 4 as stated above. As shown in table 3, the PIC value and heterozygosity of BTN2 and 3 were observed relatively

Table 2. Freguencies of genotype and size of restricted fragments for each locus of bovine butyrophilin

Loci	Genotypes	Frequencies	Fragment size (bp)
Butyrophilin 1	AA	78 (79.59%)	316
	AB	20 (20.41%)	316, 280
Butyrophilin 2	AA	21 (21.43%)	305, 263
	AB	66 (67.35%)	568, 305, 263
	BB	11 (11.22%)	568
Butyrophilin 3	AA	62 (63.27%)	576
	AB	36 (36.73%)	576, 332, 244
Butyrophilin 4	AA	76 (78.00%)	291, 282, 110
	AB	22 (22.00%)	573, 291, 282, 110

Table 3. Heterozygosity and PIC value of each locus of BTN

Loci	Frequencies		PIC	Heterozygosity
	A	B		
Butyrophilin 1	0.8980	0.1020	0.1664	0.1832
Butyrophilin 2	0.5510	0.4490	0.3724	0.4948
Butyrophilin 3	0.8163	0.1837	0.2549	0.2999
Butyrophilin 4	0.8875	0.1122	0.1794	0.1992

PIC: Polymorphism information content.

high. On the contrary, these value of BTN1 and 4 were observed relatively low. All of the PIC value and heterozygosity of four BTN loci were not over 0.5. However, taking into account of the report of Falconer and Mackay (1996), it indicated that BTN2 and BTN3 might be polymorphic markers for candidate gene analysis.

SSCP of STAT5a

Figure 3 shows the polyacrylamide gel electrophoresis of SSCP in the genomic sequence of the bovine Stat5a gene. SSCP in the SH2 domain of Stat5a gene was characterized. Homozygous allele was shown in lane 2 and 3 and heterozygous allele was shown in lane 1. PCR product was used as control in lane 4.

Table 4 shows the SSCP genotype frequency of the amplified region of STAT5a gene. Two kinds of homozygous allele were observed from 79 samples and the genotype frequencies of homotype A and B were 78.57% and 2.04%, respectively. Heterotype was observed in 19 samples. The number of genotypes and alleles have not been reported. In this study, we observed three genotypes and their frequencies as follows.

DISCUSSION

For candidate gene study, we performed two steps as follows. Firstly, we designed four oligonucleotide primer pairs of BTN and one oligonucleotide primer of STAT5a gene to amplify the specific region of the genes. Secondary, we confirmed four RFLP markers in BTN and an SSCP marker in STAT5a gene and developed convenient procedures for genotyping these polymorphic sites.

Figure 3. Polyacrylamide gel electrophoresis showing SSCP of a STAT5a locus (Lane 1: Heterotype, Lane 2: Homotype 1, Lane 3: Homotype 2: Lane 4: PCR product).

Table 4. Frequencies of genotype of bovine STAT5a

Locus	Genotypes	Frequencies
STAT5a	Homotype 1	77 (78.57%)
	Heterotype	19 (19.39%)
	Homotype 2	2 (2.04%)

STAT5a: Signal transducers and activators of transcription 5a.

In four RFLPs of butyrophilin gene, Ashwell et al. (1996) reported that they could not find any amino acid substitution except for BTN1 locus. In BTN1 locus, an A (adenine) → G (guanine) single base substitution has been identified at nucleotide position 1478, which results in a conservative amino acid substitution from lysine to arginine. However, in other BTN loci, each nucleic acid substitution has occurred in intron region. In BTN2, the nucleic acid substitution occurred in 2nd intron (Husaini et al., 1999). In BTN3 and 4, each nucleic acid substitution occurred in 2nd intron and 5th intron, respectively (Zegeye et al., 1999).

In BTN2, the PIC value and heterozygosity in 98 bulls were observed very high (0.3713 and 0.4927). In BTN3, these values were observed 0.2549 and 0.2999, respectively. Chang et al. (2001) reported the PIC value and heterozygosity of κ-casein in 98 proven and young bulls as 0.2150 and 0.2450 and those of β-lactoglobulin as 0.3544 and 0.4604, respectively. Comparing with these data reported, the PIC value and heterozygosity of BTN2 were higher than those of β-lactoglobulin and κ-casein. In

addition the PIC value and heterozygosity of BTN3 were higher than those of κ-casein.

A heterozygosity value of greater than 60%, or a PIC value exceeding 0.60, indicates a potentially usefully informative marker (Curran, 1997) but the heterozygosity in a mammalian gene rarely exceeds 0.3 (Falconer and Mackay, 1996). The reason may be because a mutation rarely occurs within gene region. Since the heterozygosity and PIC value of other two milk proteins (κ-casein and β-lactoglobulin) which currently being used for informative markers were lower than those of BTN2, BTN2 might have the possibility to be useful marker. However, since the heterozygosity and PIC value of BTN1 and 4 were near to 0.1, two BTN loci may indicate skewed allele distribution. Because of small sample size, we could not expect the distribution of four BTN loci exactly. However, comparing with the reported data (Taylor et al., 1996; Husaini et al., 1999; Zegeye et al., 1999) of BTN1 and 2 loci, this study showed similar result. Although gene frequencies of BTN3 and BTN4 have not been reported, based upon the results of this study, it might be expected that the frequency of B allele was very low.

In STAT5a, although we have not been able to confirm the mutation site sequence by sequencing, SSCP marker due to DNA substitution (single nucleotide polymorphism; SNP) occurring in SH2 domain of STAT5a gene might affect amino acid sequence of STAT5a protein. The role of the SH2 domain in STAT5a protein is very important because it regulates the specificity of cytokine-induced STAT protein activation and binding of STAT proteins with receptor occurs through interaction of SH2 domain and phosphorylated tyrosine. Since the amplified region lies within SH2 domain, SNP occurring in the amplified region may affect the function of STAT5a protein.

We could not identify the reason why two kinds of homozygous allele were observed differently (homotype1 and 2). The band of homotype1 was observed with PCR product. So we might conclude that mutation did not occur in homotype1. On the contrary homotype2 and heterotype might have one or more mutation site. The observed genotype frequency of homotype and heterotype were about 80% and 20%, respectively. However, more study should be performed to investigate the allele distribution of the amplified region (SH2 domain) of STAT5a gene. Since the genotype frequency of this allele has not been reported, we were not able to compare the allele distribution with the result of other studies.

Through this study, we could study polymorphisms of two candidate genes, bovine BTN and STAT5a gene in Korean dairy cattle. In addition to these candidate genes, more candidate gene study should be performed and the results of these study may be useful basic data for QTL analysis.

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