Identification of SNP marker in the calpain gene of the crossbred cattle in Thailand

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Abstract—Beef tenderness is one of the important component in palatability but the difficulty lie in that the phenotypic data available after slaughter. Calpain, calcium–dependent enzymes, is mainly responsible for improvement in postmortem tenderization of meat. The single nucleotide polymorphism (SNP) of the calpain gene was associated with meat tenderization in beef cattle. Thus, the objective of this study was conducted to determine the diversity of the nucleotide sequence of bovine calpain gene at 316, 530 and 4751 marker of the crossbred cattle in Thailand (Kamphaengsaen beef breed, KPS cattle) by the PCR method. The results indicated that haplotypes of the 316 and 530 markers of the KPS cattle yielded two possible haplotypes, G/G and G/A. Only animal inheriting the G/G haplotype which are the cattle number 4608, 21, KU4 46007/1, KU2 46004, KU4 46006/1 and 4701 had meat that was more tender than those inheriting the G/A haplotype. Whereas, the CC genotype affecting meat tenderness at the 4751 marker was from the KPS cattle number 4608, 21 and KU4 46007/1. The incorporation of the SNP markers were found with an increasing meat tenderness in animal number 4608, 21 and KU4 46007/1. It is therefore could be concluded that testing for the calpain gene genotyping which related to the muscle tenderness trait can be adopted by the farmer in order to locate which bull to be better use in their breeding program.

Key Words: Single Nucleotide Polymorphism, Calpain, Tenderness, Kamphaengsaen beef breed

I. INTRODUCTION

Eating satisfaction results from the interaction of many traits. Beef tenderness is a criteria trait in determining consumer palatability (Koohmaraie, Kent, Shackelford, Veiseth & Wheeler, 2002). Therefore, selection for genetic improvement of variation in beef tenderness has infrequently been analyzed. Marker assisted selection, a valuable tool, could indirectly investigate this barrier if proper markers could be proved. The micromolar calcium-activated neutral protease (*CAPN1*) gene encodes a cysteine protease, μ-calpain, is the primary enzyme in the postmortem tenderization process (Koohmaraie, 1990). Recently, Page et al. (2002) found two nonsynonymous markers in *CAPN1* that produce amino acid substitutions at position 316 (glycine/alanine) and 530 (valine/isolucine) in the protein for meat tenderness variation. Whereas, White et al. (2005) has also developed the marker 4751 that produce a transition from a cytosine to a thymine. These markers have shown an association of single nucleotide polymorphism (SNP) with meat tenderness in beef cattle. The objective of this study was conducted to determine the diversity of nucleotide sequence of bovine calpain gene at 316, 530 and 4751 marker of the crossbred cattle in Thailand (Kamphaengsaen beef breed, KPS cattle; Tavitchasri, Kanthapanit, Wajjwalku & Sethakul, 2007) by the PCR method**.**

II. METERIALS AND METHODS

A. DNA Isolation and Primer Design

Seven KPS cattle were used in this investigation and the genomic DNA was extracted from blood sample according to acid guanidine thiocyanate phenol chloroform DNA extraction method by modified Siebert and Chenchik (1993) protocal. All samples were genotyped for the calpain SNP using PCR and sequencing.

B. Primer Design

The primers designed were using sequence base on the reported sequence of calpain nucleotide sequence (GenBank accession number AF252504: exons 1 to 10 and AF248054: exons 11 to 22 and complete coding sequence) (Figure 1). The PCR approach has utilized a primer pair (P1-P2, P3-P4 and P5-P6 primers for 316, 530 and 4751 marker, respectively; Table 1) to amplify the two different alleles of a polymorphism in single PCR reaction to detected single base mutation.

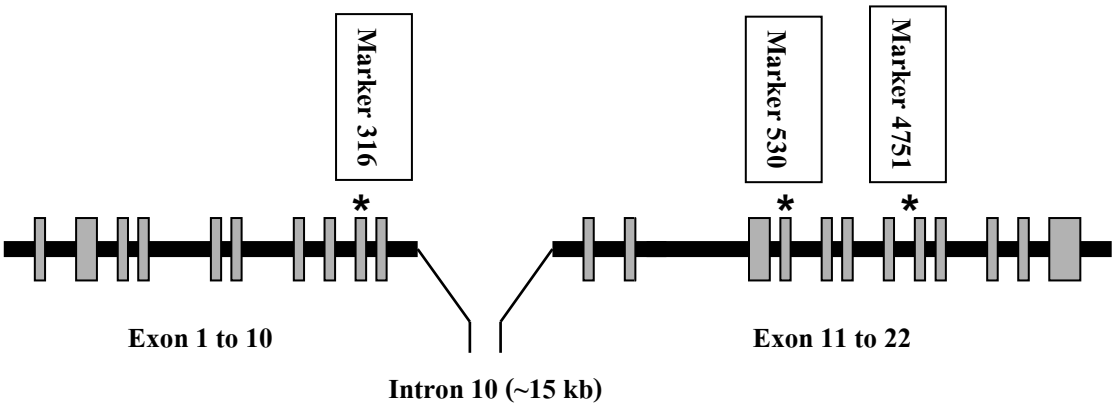

Figure 1 Genomic locations of SNP markers in calpain gene
Source : Modified from White et al. (2005)

Table 1 Primer pairs used for amplification of genomic calpain marker[1].

Primer	Sequence (5′ - 3′)	Direction	Marker	Amplicon size (bp)
P1	CTGAGCTGGCCCTCATAAGATAA	forward	316	450
P2	GTTGCGGAACCTCTGGCTCTT	reverse	316	
P3	CGAGCCCAACAAGGAAGGTG	forward	530	280
P4	TGCCTTGCTGGCTAGAGACCAA	reverse	530	
P5	CAGAGGAAGGGCTCTGGGTGA	forward	4751	185
P6	GGAGGGGTGTTCTCTGAGTGC	reverse	4751	

[1] Modified from Page et al. (2004) and White et al. (2005).

C. PCR procedure

The amplification reaction was performed in a final volume of 100 µl containing 72.5 µl of distilled water, 10 µl of 10x Taq buffer with $(NH_4)_2SO_4$, 3 µl of 50 m*M* $MgCl_2$, 2 µl of 10 m*M* dNTP, 1 µl of each forward and reverse primer, 0.5 µl of *Taq* DNA polymerase (Fermentus) and 10 µl of DNA template. The PCR was carried out in a PTC-200 Peltier Thermal Cycler machine (MJ Research) using the following conditions which were 95 ^{o}C for (5 min) for the first cycle and 95 ^{o}C (30 s) for denaturation, 62 ^{o}C (30 s) for annealing, and 72 ^{o}C (30 s) for extension, with the total of 35 cycles and 72 ^{o}C for (7 min) for the final extension. Ethidium bromide stained DNA fragments were visualized on 1.5% agarose gels.

D. Sequencing of Amplified DNA Fragments

The PCR products were sequenced by autosequencing on the ABI PRISM model 3100 Genetic Analyzer by the Genome Institute. The nucleotide sequences were aligned with the sequences from GenBank accession number AF252504 and AF248054 using ClustalW Multiple alignment (1994) and BioEdit version 7.0.5.2 (1999).

III. RESULS AND DISCUSSION

Results of PCR products were confirmed through the direct sequencing and compared with the sequences from the Genbank accession number AF252504 for marker 316 and AF248054 for marker 530 and 4751 (Figure 2).

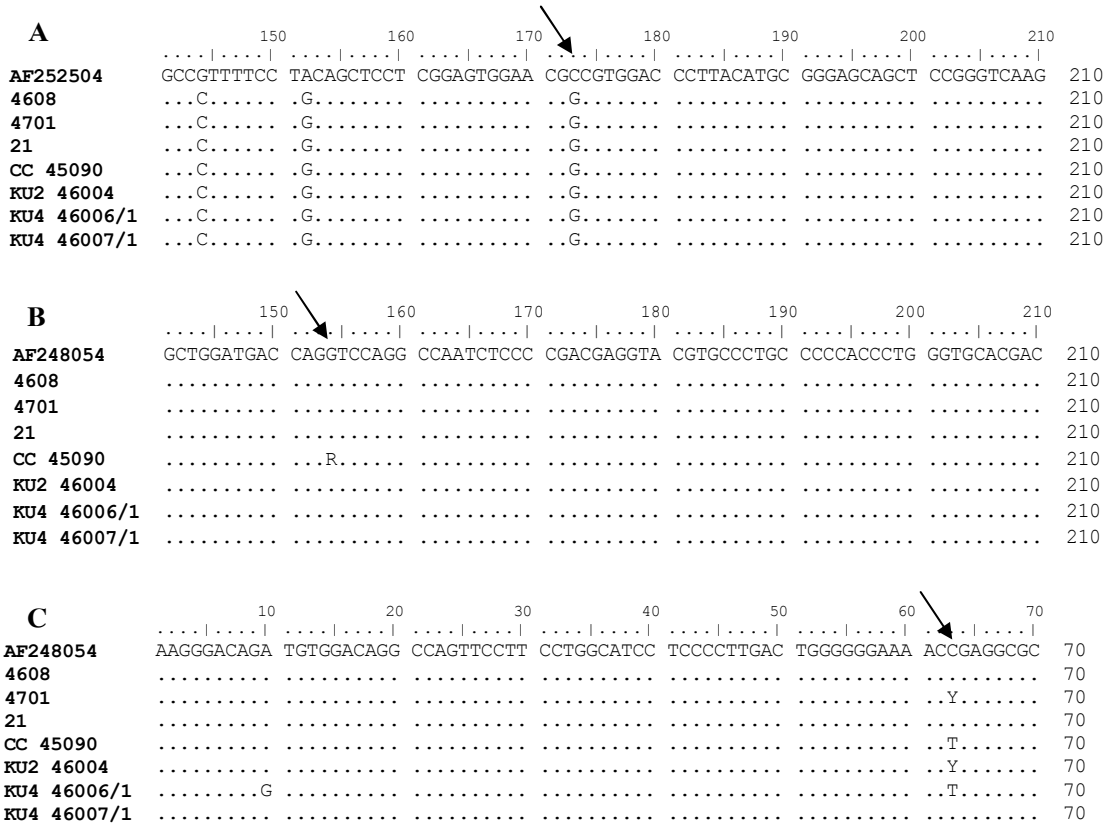

Figure 2 Nucleotide sequence alignment of the calpain gene from PCR amplification of marker 316 (A), 530 (B) and 4751 (C) of the Kamphaengsaen beef breeds compared with the sequences from GenBank accession no.AF252504 and AF248054. Denote conservation by plotting identities to a standard as a dot (.), R = G or A (purine), Y = C or T (pyrimidine). Position of each marker indicated by an arrow.

Three SNP markers were used for genotype analysis of the Kamphaengsaen cattle (KPS cattle). The marker 316 predicted amino acid changed from a guanine (G allele) to cytosine (C allele) transversion produced either glycine or alanine and an adenine (A allele) to guanine (G allele) transition produced either isoleucine or valine for the marker 530. Haplotypes depended on the allele of the marker 316/530. The haplotype coding for glycine and valine was found to be associated with decreased shear force (Page et al. 2002). The result revealed that haplotypes of the 316 and 530 markers of the KPS cattle given the two possible haplotypes, G/G and G/A, where the first allele was the 316 marker and the second allele was the 530 marker. Only animal inheriting the G/G haplotype that is the cattle number 4608, 21, KU4 46007/1, KU2 46004, KU4 46006/1 and 4701 had meat that was more tender than those inheriting the G/A haplotype (Table 2).

Table 2 Genotypes for marker 316, 530 and 4751 of the KPS cattle.

Animal	Marker		
Number	316	530	4751
4608	GG	GG	CC
21	GG	GG	CC
KU4 46007/1	GG	GG	CC
KU2 46004	GG	GG	CT
KU4 46006/1	GG	GG	TT
CC 45090	GG	GA	TT
4701	GG	GG	CT

For another marker, marker 4751, was also associated with function variation affecting tenderness in the Brahman cattle and/or crossbred descendant. The genotype CC was more tender than CT and TT

(White et al. 2005). Analysis of genotype indicated that the CC genotype affecting meat tenderness at the 4751 marker was from the KPS cattle number 4608, 21 and KU4 46007/1 (Table 2).

The SNP markers which related to the increasing meat tenderness were detected in the animal number 4608, 21 and KU4 46007/1. The combination of marker to test polymorphism might improve reliability of the meat tenderness prediction.

IV. CONCLUSION

Our research was a preliminary study for the basis of genetic marker related to the meat tenderness in commercial crossbred cattle (KPS cattle) in Thailand. Three marker genotypes of each animal were considered jointly for the prediction of beef tenderness. Analysis of genotypes revealed that the KPS cattle number 4608, 21 and KU4 46007/1 might have more tender meat and the policy for selection these cattle for breeder should be reconsidered. Therefore, the identification of these markers in the calpain gene might be useful for increased accuracy of selection and improvement rates of genetic progress for meat tenderness.

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