Polymorphisms in μ-calpain and calpastatin genes associated with tenderness in a crossbred Brahman-Angus population

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Introduction

Multiple factors, including palatability, nutritional value and safety, determine meat quality, and the importance of these traits varies depending on both the end product and the consumer profile [1]. Meat quality results from a complex relationship between features perceived by the consumer, such as tenderness. Tenderness is considered the most important quality trait in beef, and it depends on several factors, such as the amount of connective tissue and the myofibrillar protein degradation [2]. The rate of activation of μ-calpain, a proteolytic enzyme, is linked to the extent of degradation of structural proteins of myocyte [3]. Calpastatin is the specific inhibitor of the ubiquitous calcium-dependent proteases, μ-calpain and m-calpain [4].

The **objectives** of this study were to: 1) report the allele and genotype frequencies of 15 SNPs in μ-calpain and 22 SNPs in calpastain; 2) construct linkage disequilibrium (LD) blocks in each gene; 3) carry out association tests for SNP and diplotypes of LD blocks and 4) screen reported SNPs inside the associated LD-blocks to reveal possible functional SNP in those regions.

Materials and Methods

Cattle population: Cattle were classified based on their expected Angus breed composition in 6 groups: 1=100-80%; 2=79-65%; 3= 62.5% (Brangus); 4=59-40%; 5=39-20%; 6=19-0%.

Phenotype data: Warner-Bratzler Shear Force (WBSF) was measured on *Longissimus dorsi* steaks from 673 crossbreed Angus-Brahman steers.

SNP genotyping: Animals were genotyped with the 250K Bovine SNP BeadChip which included 37 SNPs in μ-calpain and calpastatin genes.

Statistical analysis: An association test was performed for each polymorphic SNP and for diplotypes created based on LD blocks using a General Lineal Model procedure (SAS Inst. Inc., Cary, NC). A Bonferroni adjustment was applied after the following model was used:

$$Y_{ijkl} = \mu + \beta_1(X_{ijkl} - X_{\ldots}) + Ai + Bj + Ck + Eijkl$$

Where: Yijkl= animal WBSF; μ = Overall mean; ß1 = Covariate Pcookloss; Ai= fixed effect genotype or fixed effect haplotype; Bj= fixed effect birth year; Ck= fixed effect breed group (1 to 6); Eijkl = random error.

Bioinformatic analysis: Three types of analyses were performed to select candidate SNPs. 1) Transcription factor binding sites analysis: MATCHTM software (5); 2) mRNA stability and folding analysis: RNAFold web server; 3) Protein analysis: SwissModel web server and Compute pI tool of ExPASy.

Results

Allele frequencies: Minor allele frequency for several SNPs was significantly different across breed groups (**Figure 1**).

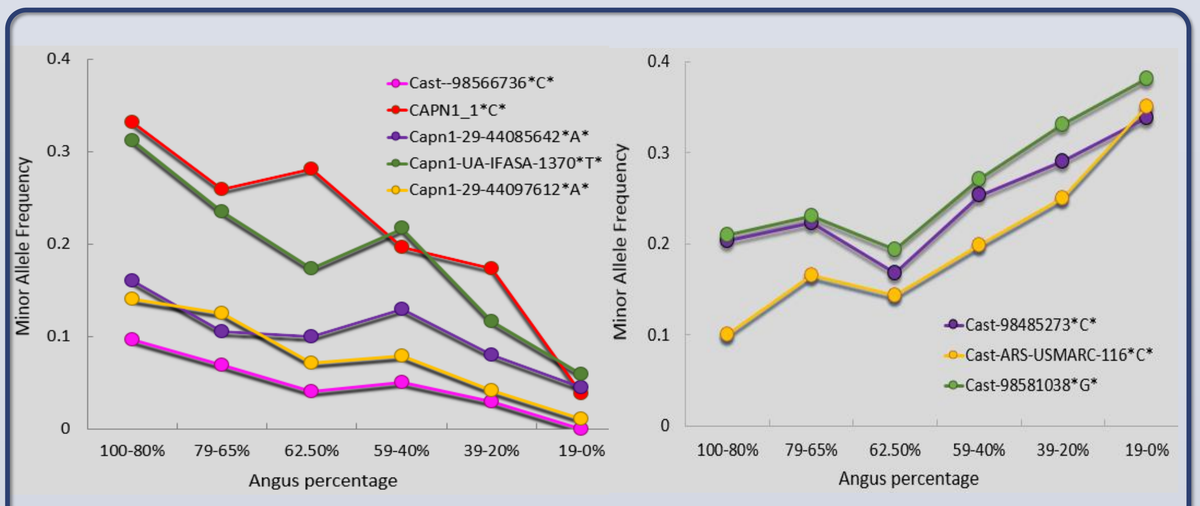

Figure 1. Minor allele frequency for Calpain (CAPN1) and calpastatin (CAST) SNPs across breed groups ranging from 100% Angus to 100% Brahman. The left panel shows SNPs with a decreasing MAF and the right panel shows SNPs with an increasing MAF as percentage Brahman increases.

LD-blocks: One LD-block was predicted in μ-calpain and three LD-blocks in calpastatin (**Figure 2**).

Association tests: One SNP in calpastatin and the third LD-block harboring this SNP were associated with WBSF. The CG-CG diplotype (4.82 ± 0.16 kg) was significantly different from TA-CG (4.40 ± 0.08 kg), and both of them were significantly different from TA-TA (4.21 ± 0.05 kg) and TG-CG (3.85 ± 0.23 kg).

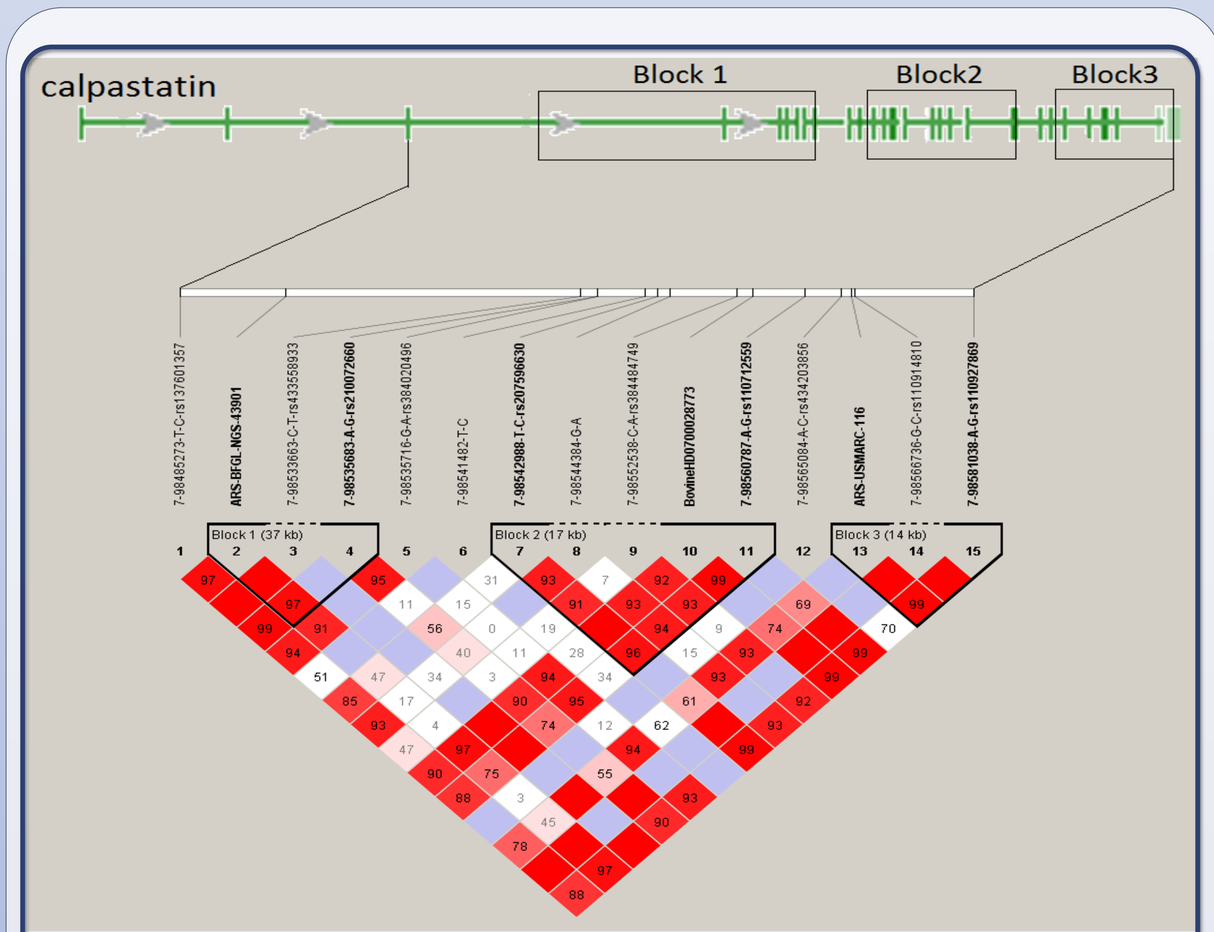

Figure 2. Location and length of the predicted LD blocks in calpastatin gene.

Screening of reported SNPs: A bioinformatic analysis of all 786 SNPs reported in the third LD-block was performed, using JAVA for selection, substitutions, transcription and translation of sequences. MATCHTM was used to predict Transcription Factor Binding Sites (TFBS) and compare the effect each SNP on these TFBS. Each mRNA sequence was analyzed with RNAFold for prediction of ΔG and folding. A protein analysis was performed with Swiss-Model server and Compute-PI/MW. Twelve SNPs modified TFBS and eight SNPs changed the ΔG value and mRNA folding. At the protein level, 13 SNPs were able to change the value of isoelectric point, and four SNPs modified protein surface.

Primer sets were designed to genotype these putative functional SNPs. None of them were polymorphic in the present population. However, other nine SNPs and three indels are located in this LD-block.

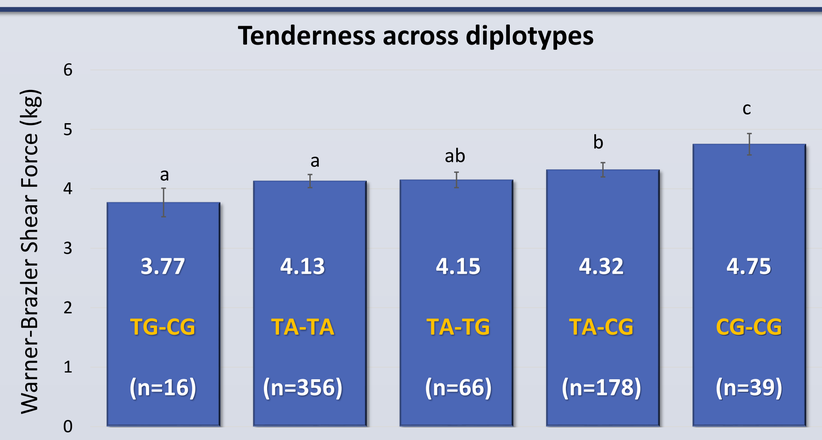

Figure 3. Average Warner-Bratzler shear force (kg) for the third LD-block diplotype of calpastatin. The third LD-block has ARSUSMARC116 and 7-98581038 as tag SNPs.

Conclusion

Minor allele frequencies for some SNPs changed drastically across breed groups. One LD-block was detected in μ-calpain; three LD-blocks were found in calpastatin. The last LD-block was associated with WBSF with the TG-CG diplotype being the associated with the most tender meat.

References

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