Genetic contribution pattern of the *CAPN1* locus over meat tenderness across post-mortem aging stages in beef cattle

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Introduction

The tenderness is the major factor associated with the eat quality of beef and has an important contribution for the consumer acceptance. The tenderization process results from a combination of ante- and post-mortem factors. Among the post-mortem processes, the proteolysis of myofibrils and connective tissues during the aging is the major contributor for the final tenderness level (Lana & Zolla, 2016). During the aging processes, several environmental and genetic factors contribute for the meat tenderness, such as breed, age, sex, and genetic variants (Frylinck et al., 2009; M. Koohmaraie & Geesink, 2006; Morgan, Wheeler, Koohmaraie, Crouse, & Savell, 1993; Shackelford et al., 1994). The μ-calpain (*CAPN1*) locus, mapped on BTA29:43400333-43427397 in the ARS-UCD 1.2 assembly of the bovine reference genome, is the main quantitative trait locus for meat quality traits across the cattle genome (Page et al., 2002; Smith, Casas, Rexroad, Kappes, & Keele, 2000). The protein μ-calpain is responsible by the major post-mortem proteolysis and tenderness development in the meat through a calcium activated cysteine protases processes (Geesink, Kuchay, Chishti, & Koohmaraie, 2006; O'Halloran, Troy, Buckley, & Reville, 1997). Currently, the genetic mechanisms related with the regulation of the tenderness across post-mortem aging are still unclear. The proper identification of these genetic mechanisms may help to identify the genetic components responsible for the tenderization process in each post-mortem stage and consequently, help to improve the genetic selection accuracy for meat tenderness in beef cattle.

Material and Methods

Two thousand two hundred sixty-seven crossbred bulls, composed by 6 major breeds (Angus, Charolais, Simmental, Piedmontese, Limousin, and Gelbvieh), from the Ontario Beef Research Center; University of Guelph (Elora, ON, Canada) were used in the present study. These animals were genotyped using the Illumina BovineSNP 50K and after quality control including autosomal chromosomes, minor allelic frequency>0.01, call rate >0.95 and departure of heterozygous from Hardy-Weinberg equilibrium>0.15, 37,564 markers were maintained. The meat tenderness was defined using the Warner-Bratzler shear force to measure the amount of force (kg) required to cut through the cooked *longissimus dorsi* in three different aged samples: 7, 14- and 21-days post-mortem (LMD7, LMD14 and LMD21, respectively). In total 1,740, 1,295, and 1,288 animals were sampled for LMD7, LMD14 and LMD21, respectively. The weighted single-step method (WssGBLUP) (Wang, Misztal, Aguilar, Legarra, & Muir, 2012), was used to estimate the SNP effects. The software AIREMLF90 was used to obtain the estimate breeding values and the solutions for SNP effects, where a single-trait model was considered including sex, herd, year, month of birth, expected breed composition and slaughter age as fixed effects, the vector of animal additive genetic effects as random effect and the vector of residual effects. The effect of each SNP was obtained using the equation proposed by Wang, Misztal, Aguilar, Legarra, & Muir (2012) in two rounds of WssGBLUP. The PostGSf90 software was used to estimate the proportion of variance explained by 1 Mega base (Mb) non-overlapping windows. The positional candidate genes within the candidate windows were annotated using the Genomic Annotation in Livestock for positional candidate Loci (GALLO) package in R as described by Fonseca, Suárez-Vega, Marras, & Cánovas (2020) using the bovine reference genome ARS-UCD 1.2 assembly.

Results and Discussion

The 1 Mb windows which the *CAPN1* gene was mapped were identified for each evaluated trait. The candidate window for LMD7, LMD14 and LMD21 was mapped in the BTA29:42445128-43424442, BTA29:42844803-43750816, and BTA29:43422582-443776600. These windows explained 4.63% (1st window for LMD7), 1.18% (5th window for LMD14) and 0.03% (65th window for LMD21) of the total genetic variance, respectively. The windows surrounding the candidate widows presented above explained a smaller percentage of the total genetic variance (data not shown). The percentage of the genetic variance explained by the 1 Mb adjacent sliding window for each marker inside the candidate windows highlight the relationship between the genetic variance and the proximity of *CAPN1* (Figure 1). Indeed, some functional candidate markers were already identified within *CAPN1* and were used to guide genomic selection in beef cattle (Casas et al., 2006; Page et al., 2002; Smith et al., 2000; White et al., 2005). It is well established that the principal mechanism of post-mortem tenderization is the proteolysis of myofibrillar proteins, with a rapid decrease in shear-force up to 10 days mainly caused by the weakening of myofibrillar structures followed by the weakening of endomysium and perimysium (Huff Lonergan, Zhang, & Lonergan, 2010; Nishimura, Liu, Hattori, & Takahashi, 1998). In addition, in lamb and beef carcasses it was demonstrate that the infusion in a solution of calcium chloride accelerated the post-mortem proteolysis and tenderization, reaching a peak within 24 hours, compared with 7-14 days in non-infused carcasses (Mohammad Koohmaraie, 1988, 1994). The μ-calpain activity during the first 7 days post-mortem shows a decreasing pattern, reaching activity levels smaller than 4% when compared with its at-death activity. These results evidence a possible higher activity of the calpain complex around the first 14-days post-mortem. Several factor might explain this higher activity of μ-calpain, such as the decline of pH, temperature, calcium availability and presence of μ-calpain inhibitors (Boehm, Kendall, Thompson, & Goll, 1998). These biological evidences help to explain the higher contribution for those windows harboring *CAPN1* for the total genetic variance in LMD7 and LMD14, but not in LMD21. On the other hand, the WssGBLUP results presented here, reinforce the hypothesis that other biological processes might be acting in the meat tenderization beyond the proteolysis cause by the calpain complex activity (Veiseth-Kent, Pedersen, Rønning, & Rødbotten, 2018).

Conclusions

Different genetic components act during the post-mortem tenderization process. The proper identification of these genetic components might help to better guide the genetic selection process for meat tenderness in beef cattle. Consequently, helping to obtain a meat with better quality standards in a shorter period. The results obtained here confirm the contribution of genetic variants in *CAPN1* locus for the meat tenderness levels in 7- and 14-days post-mortem, but not in 21 days. Consequently, indicating that other processes might be acting over the tenderization in later stages post-mortem. These processes must be further investigated in order to identify the genetic components responsible for its regulation.

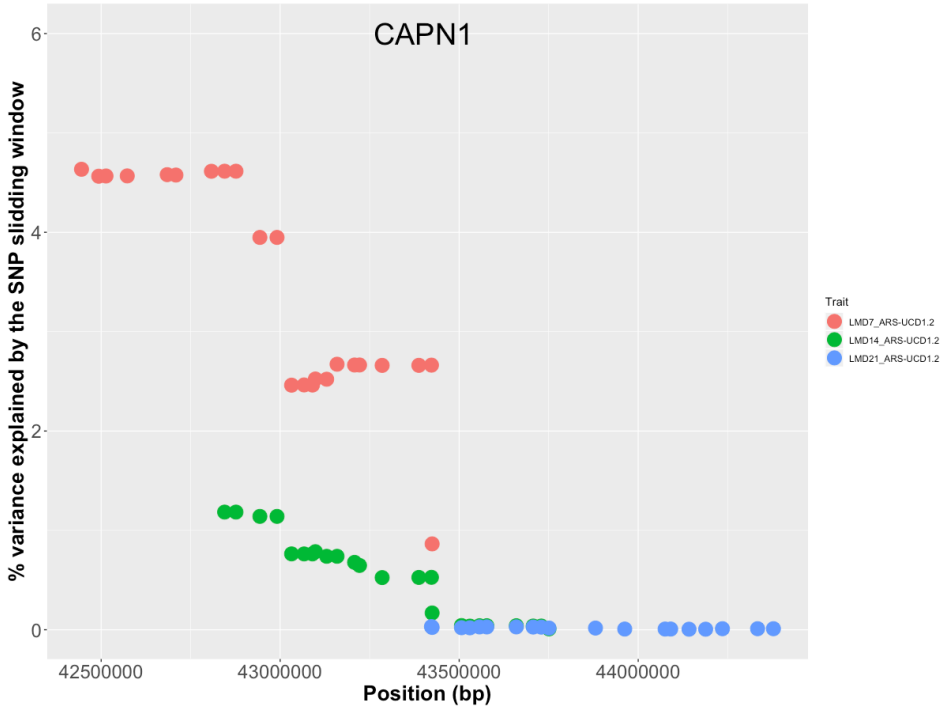

Figure 1: Percentage of the variance explained by the 1 Mb adjacent sliding window for each marker inside the candidate windows for LMD7 (red), LMD14 (green), and LMD21 (blue). The *CAPN1* gene symbol is placed in the genomic position within the candidate windows where the gene is mapped.

References

Boehm, M. L., Kendall, T. L., Thompson, V. F., & Goll, D. E. (1998). Changes in the Calpains and Calpastatin During Postmortem Storage of Bovine Muscle. *Journal of Animal Science*. https://doi.org/10.2527/1998.7692415x

Casas, E., White, S. N., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., … Smith, T. P. L. (2006). Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. *Journal of Animal Science*. https://doi.org/10.2527/2006.843520x

Fonseca, P. A. S., Suárez-Vega, A., Marras, G., & Cánovas, A. (2020). GALLO: An R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci. *GigaScience*. https://doi.org/10.1093/gigascience/giaa149

Frylinck, L., van Wyk, G. L., Smith, T. P. L., Strydom, P. E., van Marle-Köster, E., Webb, E. C., … Smith, M. F. (2009). Evaluation of biochemical parameters and genetic markers for association with meat tenderness in South African feedlot cattle. *Meat Science*. https://doi.org/10.1016/j.meatsci.2009.07.016

Geesink, G. H., Kuchay, S., Chishti, A. H., & Koohmaraie, M. (2006). μ-calpain is essential for postmortem proteolysis of muscle proteins. *Journal of Animal Science*. https://doi.org/10.2527/jas.2006-122

Huff Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle - Lessons on mechanisms of meat tenderization. *Meat Science*. https://doi.org/10.1016/j.meatsci.2010.05.004

Koohmaraie, M., & Geesink, G. H. (2006). Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*. https://doi.org/10.1016/j.meatsci.2006.04.025

Koohmaraie, Mohammad. (1988). The role of endogenous proteases in meat tenderness. *Proc. Recip. Meat Conf*, *41*(89), 100.
Koohmaraie, Mohammad. (1994). Muscle proteinases and meat aging. *Meat Science*. https://doi.org/10.1016/0309-1740(94)90036-1
Lana, A., & Zolla, L. (2016). Proteolysis in meat tenderization from the point of view of each single protein: A proteomic perspective. *Journal of Proteomics*. https://doi.org/10.1016/j.jprot.2016.02.011
Morgan, J. B., Wheeler, T. L., Koohmaraie, M., Crouse, J. D., & Savell, J. W. (1993). Effect of castration on myofibrillar protein turnover, endogenous proteinase activities, and muscle growth in bovine skeletal muscle. *Journal of Animal Science*. https://doi.org/10.2527/1993.712408x
Nishimura, T., Liu, A., Hattori, A., & Takahashi, K. (1998). Changes in Mechanical Strength of Intramuscular Connective Tissue during Postmortem Aging of Beef. *Journal of Animal Science*. https://doi.org/10.2527/1998.762528x
O'Halloran, G. R., Troy, D. J., Buckley, D. J., & Reville, W. J. (1997). The role of endogenous proteases in the tenderisation of fast glycolysing muscle. *Meat Science*. https://doi.org/10.1016/S0309-1740(97)00046-6
Page, B. T., Casas, E., Heaton, M. P., Cullen, N. G., Hyndman, D. L., Morris, C. A., … Smith, T. P. L. (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *Journal of Animal Science*. https://doi.org/10.2527/2002.80123077x
Shackelford, S. D., Koohmaraie, M., Cundiff, L. V., Gregory, K. E., Rohrer, G. A., & Savell, J. W. (1994). Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. *Journal of Animal Science*. https://doi.org/10.2527/1994.724857x
Smith, T. P. L., Casas, E., Rexroad, C. E., Kappes, S. M., & Keele, J. W. (2000). Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. *Journal of Animal Science*. https://doi.org/10.2527/2000.78102589x
Veiseth-Kent, E., Pedersen, M. E., Rønning, S. B., & Rødbotten, R. (2018). Can postmortem proteolysis explain tenderness differences in various bovine muscles? *Meat Science*. https://doi.org/10.1016/j.meatsci.2017.11.011
Wang, H., Misztal, I., Aguilar, I., Legarra, A., & Muir, W. M. (2012). Genome-wide association mapping including phenotypes from relatives without genotypes. *Genetics Research*. https://doi.org/10.1017/S0016672312000274
White, S. N., Casas, E., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., … Smith, T. P. L. (2005). A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of Bos indicus, Bos taurus, and crossbred descent. *Journal of Animal Science*. https://doi.org/10.2527/2005.8392001x