

New Alleles in the Calpastatin Gene Associated with Improved Tenderness in Pork

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Summary and Implications

Suggestive quantitative trait loci (QTL) affecting average Instron force and other tenderness measures were mapped to pig chromosome 2 (SSC 2) by using a three-generation intercross between Berkshire x Yorkshire (B x Y) pigs. Based on the QTL location, the Calpastatin (*CAST*) gene was considered to be a good candidate for the observed effects. Differences in the *CAST* gene sequences of the members of the B x Y intercross were analyzed. Two missense substitutions (changes affecting proteins) were identified. To test the hypothesis that at least one of these mutations was associated with differences in tenderness, individuals from F₂ B x Y, and from a Duroc x Yorkshire (D x Y) intercross, were genotyped for the candidate gene differences and association studies were performed. The results provide significant support for the presence of new economically important alleles (gene forms) of the *CAST* gene affecting the Instron/Shear Force and the resulting meat quality. The significant effect of these markers on tenderness measures, will add them to the list of already discovered Halothane and RN markers, as a set of molecular tools developed to improve pork quality.

Introduction

The identification of some suggestive quantitative trait loci (QTL) for tenderness traits, including Instron Force (1), in a region where *CAST* gene should be located, suggested that new allelic (genetic) variation in this gene might be responsible for the observed effects. Calpastatin (*CAST*) is a specific inhibitor of calpains, a Ca²⁺-activated protease family, considered to be the major cause of initiation of myofibrillar protein degradation in living muscle (2). There are suggestions that calpains play an important role in postmortem tenderization of skeletal muscle due to the degradation of key myofibrillar and associated proteins (3). We report the presence of new economically important alleles of the *CAST* gene affecting the Instron/Shear

Force and related meat quality traits of pigs such as firmness and juiciness.

Materials and Methods

We generated an intercross between Berkshire and Yorkshire (B x Y) pig breeds yielding 525 F₂ offspring and used this pedigree to map QTL for meat quality (1). In this cross, the Berkshire breed was chosen because it is regarded as having very good meat quality, including ultimate pH, color, water holding capacity, and tenderness.

Based on the *CAST* pig gene sequence we searched for polymorphisms in the members of the B x Y reference family as possible causes of the observed variations in Instron force and related meat quality traits. We used a previously identified mutation – *CAST MspI* – (4) to map *CAST* gene to the B x Y family linkage map.

Associations between each of the missense substitutions and tenderness measures, including Instron force, were tested in individuals from F₂ B x Y and also in D x Y intercross for Warner - Bratzler Shear Force. Standard statistical analyses were performed.

Results and Discussion

Marker development and linkage mapping

Several suggestive QTL were detected on SSC2 (1) in the region where the *CAST* gene should be located. These included QTL for Instron force, chew, and tenderness scores that could be affected by the variation in *CAST* gene. By sequencing the entire coding region of the *CAST* gene in members of the B x Y family, we identified two missense mutations (*CAST Hpy188I* and *CAST PvuII*). Using the previously identified *CAST MspI* mutation, we mapped the *CAST* gene in the B x Y linkage map to a position below the peak(s) of the QTL for Instron force, chew, and tenderness scores.

Association Analysis of Commercial Populations

An association analysis on the B x Y F₂ animals showed significant effects for both polymorphisms tested on Instron force and in some of the traits associated with it, such as firmness and juiciness (data shown only for *CAST Hpy188I*, Table 1). The *CAST Hpy188I-11* genotype is favorable in terms of meat quality and is associated with lower firmness, lower chew score, and less Instron force and higher tenderness and juiciness. In the D x Y cross, although numbers of animals for the 22 genotypes are limited, the same 11 genotypes for both polymorphisms are associated with lower Warner-Bratzler Shear Force (Table 2).

The results reported in this work provide evidence to suggest the presence of new alleles of the *CAST* gene affecting meat quality traits. This conclusion is based on the following: 1) the suggested effect of *CAST* on in postmortem tenderization of skeletal muscle (3); 2) the observation of several QTL for Instron force and related meat quality traits on *SSC2* in a region where *CAST* is located in the B x Y family; and 3) the associations between the *CAST* substitutions and tenderness measures in B x Y F2 but also in a different intercross (D x Y).

It remains to be further demonstrated if the revealed effects are caused by these substitutions alone, or due to linkage disequilibrium. A combined use of the polymorphisms discovered could have an important potential to improve overall meat quality and hence the economic value for pork supply chain and quality products for consumers.

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Table 1. Association results between genotypes of *CAST Hpy188I* and meat quality traits in BxY F₂ animals^{A, B}.

| Traits | Genotype | | | p |
|--------------------|---------------------|-------------------|-------------------|-------|
| | 11 | 12 | 22 | |
| Firmness | 3.21 ^{e,c} | 3.44 ^f | 3.43 ^d | 0.001 |
| Juiciness | 6.23 ^a | 6.05 | 5.76 ^b | 0.05 |
| Tenderness | 8.01 ^a | 7.74 ^b | 7.75 | 0.11 |
| Chew score | 2.32 | 2.51 | 2.54 | 0.11 |
| Instron force (kg) | 4.39 ^a | 4.45 ^a | 4.63 ^b | 0.05 |

^A n=136 (11), 228-233 (12), and 129-130 (22).

^B Significant differences: a-b, p<.05; c-d, p<.005; e-f, p<.0005.

Table 2. Association results between the genotypes of *CAST* substitutions and Warner–Bratzler Shear force at in D x Y animals^A.

| Polymorphisms | Aging day | Genotypes | | | p |
|----------------|-----------|--------------------------|--------------------------|-------------------------|-------|
| | | 11 | 12 | 22 | |
| <i>Hpy188I</i> | 3 | 3.08 (.22) ^a | 3.43 (.20) | 4.85 (.59) ^b | 0.03 |
| | 5 | 2.93 (.20) ^c | 3.26 (.01) ^c | 5.03 (.53) ^d | 0.005 |
| n | | 27 | 31 | 6 | |
| <i>PvuII</i> | 3 | 3.47 (0.24) | 3.86 (0.21) | 2.92 (0.92) | 0.23 |
| | 5 | 3.08 (0.21) ^c | 3.80 (0.19) ^d | 3.88 (0.81) | 0.03 |
| n | | 40 | 22 | 2 | |

^A Significant differences: a-b, p< 0.05; c-d, p< 0.01.