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

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Abstract
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

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

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ASL-R1804

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 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 HpyI188I 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 HpyI188I, Table 1). The CAST HpyI188I-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).

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.
The results reported in this work provide evidence to suggest the presence of new alleles of the \textit{CAST} gene affecting meat quality traits. This conclusion is based on the following: 1) the suggested effect of \textit{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 \textit{CAST} is located in the B x Y family; and 3) the associations between the \textit{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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We thank J. Bastiaansen and G.S. Plastow, Sygen International, Berkeley, CA, and J. Helm, Y. Zhang and H. Thomsen, Iowa State University, for technical support. This work was financially supported by Sygen International, PIC USA, the Iowa Agriculture and Home Economics Experimental Station, Ames and from Hatch and State of Iowa funds.

\textbf{References}


Table 1. Association results between genotypes of \textit{CAST Hpy188I} and meat quality traits in BxY F$_2$ animals$^A$.\textsuperscript{,}B.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genotype</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Firmness</td>
<td>3.21$^{e,c}$</td>
<td>3.44$^d$</td>
</tr>
<tr>
<td>Juiciness</td>
<td>6.23$^a$</td>
<td>6.05$^f$</td>
</tr>
<tr>
<td>Tenderness</td>
<td>8.01$^a$</td>
<td>7.74$^b$</td>
</tr>
<tr>
<td>Chew score</td>
<td>2.32$^b$</td>
<td>2.51$^d$</td>
</tr>
<tr>
<td>Instron force (kg)</td>
<td>4.39$^a$</td>
<td>4.45$^a$</td>
</tr>
</tbody>
</table>

$^A$ 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 \textit{CAST} substitutions and Warner–Bratzler Shear force at in D x Y animals$^A$.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Aging day</th>
<th>Genotypes</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Hpy188I}$</td>
<td>3</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>n</td>
<td>3</td>
<td>3.08 (.22)$^a$</td>
<td>3.43 (.20)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.93 (.20)$^c$</td>
<td>3.26 (.01)$^c$</td>
</tr>
<tr>
<td>$\text{PvuII}$</td>
<td>3</td>
<td>3.47 (0.24)</td>
<td>3.86 (0.21)</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>3.08 (0.21)$^a$</td>
<td>3.80 (0.19)$^d$</td>
</tr>
</tbody>
</table>

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