

New Alleles in the “RN gene” Associated with Low Glycogen Content in Pig Skeletal Muscle and Improved Meat Quality

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Summary and Implications

Several quantitative trait loci (QTL) affecting muscle glycogen content and related meat quality traits were mapped to pig chromosome 15 (SSC 15) by using a three-generation intercross between Berkshire x Yorkshire pigs. Based on the QTL location, the *PRKAG3* (protein kinase AMP-activated, γ_3 subunit) or the RN (Rendement Napole) gene, as it is popularly known, was considered to be a good candidate for the observed effects. Differences in the *PRKAG3* gene sequences of the founder animals of the intercross were analyzed. The RN mutation previously reported was not present in the cross but three missense substitutions (changes affecting proteins) were identified. To test the hypothesis that at least one of these mutations was associated with differences in meat quality, more than 1,800 animals from several unrelated commercial lines were genotyped for the candidate gene differences and an association study was performed. The results provide strong support for the presence of new economically important alleles (gene forms) of the *PRKAG3* gene affecting the glycogen content in the muscle and the resulting meat quality. Because of their prevalence in the more common commercial breeds, the potential implications for the pig industry and consumers are probably more important than the original discovery of the RN mutation.

Introduction

The recent discovery (1) of a nonconserved substitution in the *PRKAG3* gene has explained the dominant mutation (denoted RN⁻) that accounted for important differences in meat quality and processing yield in the Hampshire pig breed (2,3). A mutation (R200Q – changing arginine to glutamine) in the *PRKAG3* results in a 70% increase in glycogen in muscle in RN⁻ homozygous and heterozygous

animals that then results in the observed lower muscle pH 24 h after slaughter, reduced water holding capacity in the muscle and much lower yield of a cured cooked ham product.

The identification of QTL for meat quality traits in the region of *PRKAG3* gene in an rn⁺ resource population (4) suggested that new allelic (genetic) variation in this gene may be responsible for the observed effects. We report the presence of new economically important alleles of the *PRKAG3* gene affecting the glycogen content in muscle and several meat quality traits of pigs which include ultimate pH and color measures and which are correlated with water holding capacity, drip loss, tenderness, and cooking loss.

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 (4). In this cross, the Berkshire breed was chosen because it is regarded as having very good meat quality, particularly in terms of pH, color, water holding capacity, and tenderness.

Based on the *PRKAG3* pig gene sequence we searched for additional polymorphisms in the founders of B x Y reference family as possible causes of the observed variations in glycogen measures and meat quality traits. One of the polymorphisms was used to map *PRKAG3* gene to the B x Y family linkage map.

Associations between each of the three nonsynonymous substitutions and meat quality traits were tested in several commercial populations (Landrace, Large White, Berkshire, Duroc, and Duroc Synthetic). Standard statistical analyses were performed. No measures of glycogen or glycolytic potential were obtained. However, the measures of color and pH phenotypic traits used are common industry measures of meat quality that are indirectly correlated with glycogen and glycolytic potential.

Results

Marker development and linkage mapping

Several significant QTL were detected on SSC15 (4) in the region where the *PRKAG3* gene was located (1). These included QTL for average glycogen content and glycolytic potential that have been reported (1) to be affected by the *PRKAG3* 200Q allele as well as the traits 24 h ham and loin pH and 24 h loin Hunter L values (light reflectance).

Interestingly, for these QTL, the favorable allele has an additive effect (the RN⁻ mutation is dominant) and was derived predominantly from the Berkshire breed as expected (4). The *PRKAG3* gene was the unique candidate gene in this area. All the founder animals were homozygotes for the rn⁺ allele. By sequencing the entire coding region of the *PRKAG3* gene in B x Y family founders, we identified three missense mutations (I199V, G52S and T30N). Using the I199V substitution we mapped the *PRKAG3* gene in the B x Y linkage map to a position below the broad peak(s) of the QTL for glycogen, lactate, and glycolytic potential (Figure 1).

Association Analysis of Commercial Populations

For all three substitution sites, the Berkshire line had a higher frequency for the genotypes associated with low glycogen content (and higher meat quality) in skeletal muscle based on the B x Y F₂ data (data not shown). The *PRKAG3* mutations and their associations with meat quality were tested for each of the five commercial lines and also across all of the lines. The association study revealed that the I199V substitution has largest effects across the lines (Table 1) and also within lines. For this substitution the associations were highly significant ($p < 0.0005$) for all of the meat quality traits used in this study when analyzed across lines. Significant associations with at least one of the traits were revealed for the same substitution within each of the individual commercial pig lines. In the across lines analysis and most of the individual line results, the effects were in the same direction for all traits with allele 199I being the favorable allele for high meat quality.

Significant effects, but smaller compared with the I199V, were also found for G52S and T30N substitutions in some of the analyzed traits and populations.

Discussion

The results reported in this work provide important evidence to suggest the presence of new alleles of the *PRKAG3* gene affecting meat quality traits. This conclusion is based on three points: 1) the known effect of *PRKAG3* alleles, rn⁺ and RN⁻ on meat quality; 2) the observation of several QTL for related meat quality traits on SSC15 in the region where *PRKAG3* is located in the B x Y family (which is rn⁺); and 3) the association between the *PRKAG3* substitutions and meat quality traits in several unrelated commercial pig lines.

Association analyses of the individual substitutions revealed that, of the three studied here, the I199V substitution showed the most significant and largest differences in meat quality traits. Allele 199I was found to be associated with a lower level of

glycogen, lactate, and glycolytic potential, higher ham and loin pH, and better color scores.

Although the effects of the substitutions reported here on the measures of meat quality are of lesser magnitude than those associated with the dominant RN⁻ mutation, they are of importance both biologically and economically. In particular, these alleles are segregating in all of the commercial lines and breeds analyzed to date in contrast to the RN⁻ mutation, which is associated only with the Hampshire breed and has limited use in most pork production programs.

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Table 1. Association results between the genotypes at I199V substitution site of the *PRKAG3* gene and meat quality traits across five commercial breeds.

| Traits | 199I/199I | 199I/199V | 199V/199V |
|----------------|-------------------------|---------------------------|---------------------------|
| Ham pH | 5.81 (.01) ^e | 5.74 (.01) ^{f,e} | 5.71 (.01) ^f |
| Loin pH | 5.78 (.01) ^e | 5.74 (.01) ^{f,e} | 5.71 (.01) ^f |
| Ham Minolta L | 44.9 (.37) ^e | 46.5 (.27) ^f | 46.9 (.26) ^f |
| Loin Minolta L | 44.2 (.26) ^e | 44.7 (.18) ^a | 45.2 (.18) ^{f,b} |
| Ham Minolta b | 3.63 (.14) ^e | 4.30 (.10) ^{f,e} | 4.71 (.10) ^f |
| Loin Minolta b | 3.15 (.08) ^e | 3.31 (.06) ^c | 3.49 (.06) ^{f,d} |

Significant differences: a-b, $p < 0.05$; c-d, $p < 0.005$; e-f, $p < 0.0005$.

Figure 1. F-ratio curves for evidence of QTL associated with meat quality for SSC 15. The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Arrows on the x-axis indicate the position where a marker was present. Three lines are provided for 5% chromosome-wise (-----) and 5% genome-wise (———).

