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# Association of SNPs and haplotypes in adiponectin and adiponectin receptors with pig meat quality traits

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**ABSTRACT:** Adiponectin is mainly produced by the adipose tissue and its serum concentration is inversely correlated with body fat. Previous studies identified polymorphisms in the porcine *ADIPOQ, ADIPOR1* and *ADIPOR2* genes.Some of these are associated with carcass and reproductive traits. Our objective was to look for potential associations between these previously identified SNPs and various loin quality traits in Duroc, Yorkshire and Landrace barrows. Landrace pigs with the *ADIPOQ* GC and GX haplotypes had smaller Minolta L* values when compared with the AC haplotype ($P<0.05$), whereas the GC and GX animals had lower drip loss values than AC pigs ($P<0.05$). In Duroc, the *ADIPOR2* GCG haplotype showed higher drip loss and lower lactate dehydrogenase activity when compared with GCA pigs ($P<0.05$). Our results demonstrate that selecting for specific *ADIPOQ* and *ADIPOR2* haplotypes may positively affect the *longissimus* meat colour, water holding capacity and glycolytic potential in pigs.
**Key Words:** Adiponectin**;** Meat quality traits**;** Pig Polymorphisms

## Introduction

Adiponectin (ADIPOQ) is an adipokine that is mainly produced and secreted by adipose tissue. Unlike most other adipokines, its serum concentration is inversely correlated with body mass index and visceral fat mass. Adiponectin plays a key role in the regulation of fatty acid oxidation, glucose metabolism and insulin sensitivity (Brochu-Gaudreau et al. (2010)). Adiponectin action is mediated through two seven transmembrane receptors (ADIPOR1 and ADIPOR2) that are expressed in several peripheral tissues, including skeletal muscle (Lord et al. (2005)). Previous results have demonstrated that adiponectin can induce gene expression and cell differentiation of C2C12 murine muscle cells (Fiaschi et al. (2009)) and that it may be involved in skeletal muscle protein synthesis and degradation (Yamauchi et al. (2001)). Polymorphisms were detected in the porcine *ADIPOQ, ADIPOR1* and *ADIPOR2* genes, and some of these were found to be associated with pig carcass (Cieslak et al. (2012); Stachowiak et al. (2009); Dall'Olio et al. (2009); Dai et al. (2006)) and reproductive traits (Houde et al. (2008)). However, no one has yet investigated for possible associations between these polymorphisms and meat quality traits in livestock. Therefore, the objective of our study was to verify the presence of associations between polymorphisms previously identified by our research group (Houde et al. (2008)) and various meat quality traits collected on the *longissimus* muscle of purebred Duroc, Yorkshire and Landrace barrows.

## Materials and Methods

**Animals and data.** A total of 701 purebred barrows from 16 breeding herds across Canada entered the Deschambault test station (Quebec). These pigs are from 2 independent station trials conducted in 2010. Animals included Duroc (DD, n = 214), Landrace (LL, n = 216) and Yorkshire (YY, n = 271) breeds (2 castrates/litter). Pigs were fed *ad libitum* and were slaughtered at a targeted live weight of 120 kg. Carcasses were tracked individually at the plant to allow *longissimus* muscle sampling immediately after slaughter and meat quality measurements 24 h after slaughter. Meat quality traits used for association analyses were: pH 24 h, colour assessed through L*, a* and b* reflectance values (Minolta CR-300), drip loss % (EZ-DripLoss funnels), shear force (texturometer), intramuscular fat (IMF) content (% of wet sample), glycolytic potential (µmol of lactate equivalent/g tissue; 2([glycogen] + [glucose] + [glucose 6 phosphate] + [lactate])), lactate dehydrogenase (LDH, IU/g tissue; glycolytic metabolism indicator) and citrate synthase (CS, IU/g tissue; oxidative metabolism indicator).

**Genotyping**. Genomic DNA was extracted from *longissimus* muscle samples by DNA LandMarks (Saint-Jean-sur-Richelieu, QC, Canada). A total of 633 pigs were genotyped (n = 188 DD, n = 196 LL and n = 249 YY) using PCR-RFLP or TaqMan single nucleotide polymorphism (SNP) assays as reported in Houde et al (2008). From that number, animal performances and meat quality traits information were available on 630 pigs. Genotyping was performed for 8 SNPs previously identified in the porcine *ADIPOQ* (c.178G>A, c.*300A>G, c.*1094_1095insC and c.*1779A>C), *ADIPOR1* (c.*129A>C) and *ADIPOR2* (c.*112G>A, c.*295G>C and c.*1455G>A) genes (Houde et al. 2008).

**Construction of haplotypes**. Haplotypes were constructed using the PHASE software (Stephens and Donnelly (2003)). SNPs with minor allele frequency (MAF) across breed of less than 5% were not included in the haplotype construction. Therefore, 2 SNPs were conserved for *ADIPOQ* (c.178G>A and c.*1094_1095insC) and 3 SNPs for *ADIPOR2* (c.*112G>A, c.*295G>C and c.*1455G>A) for haplotypes construction.

**Statistical analyses.** Association analyses between meat quality traits and ADIPOQ, *ADIPOR1* and *ADIPOR2*

genotypes and haplotypes were performed using the SAS MIXED procedure (2002). The statistical model used for SNP analyses included the fixed effects of slaughter date, SNPs and carcass weight as covariate. Association analyses with haplotypes included the fixed effect of slaughter date, number of copies of each haplotype and carcass weight as a covariate. Analyses were performed within breed. False discovery rate (FDR) was used to account for multiple testing (Benjamini and Hochberg (1995)).

## Results and Discussion

**Genotype and haplotype frequencies.** Minor allele frequencies (MAF) across breeds were 0.06, 0.01, 0.49 and 0.01 for *ADIPOQ* c.178G>A, c.*300A>G, c.*1094_1095insC and c.*1779A>C SNPs, respectively. The *ADIPOR1* c.*129A>C MAF was 0.02 and *ADIPOR2* MAF were 0.26, 0.20 and 0.25 for c.*112G>A, c.*295G>C and c.*1455G>A SNPs, respectively. SNPs with MAF ≤ 0.05 were excluded from association analyses. SNPs with a MAF ≤ 0.05 were excluded from haplotype construction, resulting in 2 conserved SNPs for *ADIPOQ* (c.178G>A and c.*1094_1095insC) and 3 SNPs for *ADIPOR2* (.*112G>A, c.*295G>C and c.*1455G>A). For *ADIPOQ*, 3 of the 4 possible haplotypes were observed across breeds with frequencies of 0.43, 0.51 and 0.06 for haplotypes GC, GX and AC, respectively. The letter "X" corresponds to the absence of a "C" in SNP c.*1094_1095insC. For *ADIPOR2*, 6 of the 8 possible haplotypes were observed. Frequencies were 0.29, 0.25, 0.25 and 0.20 for the *ADIPOR2* GCG, ACG, GCA and GGG haplotypes, respectively. There were only 4 and 2 pigs for the AGG and ACA haplotypes, respectively. Therefore these 2 haplotypes were excluded from association analyses.

**SNP association analyses.** Significant associations were observed between the *ADIPOQ* c.178G>A SNP and *longissimus* colour L* ($P < 0.05$) and drip loss % ($P < 0.01$) in LL pigs, with GG having smaller L* values than AA and GA animals. Pigs with the GG genotype also had the lowest drip loss values. An association was observed between the *ADIPOQ* c.*1094_1095insC SNP and drip loss % ($P < 0.05$) in LL pigs, with the highest drip loss values being observed in CC animals, when compared with CX and XX pigs. A significant association was also observed between the *ADIPOR2* c.*1455G>A SNP and lactate dehydrogenase ($P < 0.01$) in DD pigs, with the AG genotype showing lower activity when compared with AA animals. The *ADIPOR2* c.*112G>A SNP was found to affect all 3 colour parameters ($P < 0.05$) in YY pigs, with AA genotype having the highest L* values and GG pigs the highest a* values. Heterozygous AG pigs had lower b* values, when compared with the AA and GG genotypes.

**Haplotypes association analyses.** Significant associations between meat quality traits and *ADIPOQ* and *ADIPOR2* haplotypes are presented in Tables 1 and 2. The *ADIPOQ* haplotype associations were only observed in the LL breed, but there were only 3 DD and no YY pigs with the AC haplotype. Animals with the *ADIPOQ* haplotypes GC and GX had smaller L* values than animals with the AC haplotype ($P < 0.05$). There was also a significant association between *ADIPOQ* haplotypes and b* colour values, with smaller b* values being observed in GX than in AC pigs. There is no manuscript yet reporting an association between meat colour parameters and *ADIPOQ* SNPs in livestock. In the current study, an association was observed between *ADIPOQ* haplotypes and drip loss % ($P < 0.01$). In this case, GC and GX haplotypes had lower drip loss values when compared with the AC haplotype ($P < 0.05$). Therefore, animals with the GC and GX haplotypes may have better water holding capacity in the *longissimus* muscle. Interestingly, polymorphisms identified in exon 1 of the duck *ADIPOQ* gene were found to be associated with water holding capacity (Zhang et al., (2010)). The *ADIPOQ* c.178G>A SNP is also located in exon 1 and results in a valine-to-isoleucine substitution. This mutation is located in the adiponectin collagenous domain, which is known to play a key role in the association of adiponectin trimmers to form higher-order structures or multimers that are observed in blood. Thus, any mutation located in this collagenous domain has the potential to affect the formation of higher-order structures, which may impact on adiponectin biological activity. In DD pigs, the *ADIPOR2* GCG haplotype showed higher drip loss values and lower LDH activity when compared with the GCA haplotype ($P < 0.05$). Therefore, animals with the GCA haplotype may experience a shift in favour of glycolytic muscle fibres in the *longissimus* muscle. Interestingly, Ingelsson *et al.* (2009) found an inverse association between circulating adiponectin concentrations and the proportion of type IIb glycolytic muscle fibres. Thus, it will be of interest to determine whether *ADIPOR2* GCG and GCA haplotypes show differences in the number of IIb muscle fibres. In this study, YY pigs with the *ADIPOR2* ACG haplotype had greater L* values than GCG and GGG animals ($P < 0.05$). Moreover, GCG YY pigs had greater L* values, when compared with GGG animals.

**Table 1. Contrast between *ADIPOQ* haplotypes for different meat quality traits in LL pigs.**

| Haplotype contrast | Traits | | |
|---|---|---|---|
| | Minolta L* (**) | Minolta b* (**) | Drip loss % (***) |
| GC *vs* GX | 0.11±0.32 | 0.15±0.12 | 0.43±0.26 |
| GC *vs* AC | **-1.31±0.44** | -0.25±0.16 | **-0.93±0.36** |
| GX *vs* AC | **-1.42±0.39** | **-0.40±0.14** | **-1.36±0.32** |

Haplotype estimates in bold character are significantly different ($P < 0.05$) from each other. (**), (***)Significant associations at FDR level of 0.05 and 0.01, respectively.

**Table 2. Contrast between *ADIPOR2* haplotypes for different meat quality traits in DD and YY pigs.**

| Haplotype contrast | Traits | | |
|---|---|---|---|
| | Drip loss % (**) (DD) | LDH (**) (DD) | Minolta L* (**) (YY) |
| GCG *vs* ACG | -- | -- | **-0.51±0.22** |
| GCG *vs* GCA | **0.92±0.32** | **-126.17±40.53** | -0.21±1.31 |
| GCG *vs* GGG | -- | -- | **1.23±0.42** |
| ACG *vs* GGG | -- | -- | **1.74±0.41** |

Haplotype estimates in bold character are significantly different ($P < 0.05$) from each other. (**)Significant associations at FDR level of 0.05. LDH, lactate dehydrogenase activity.

Associations between studied SNPs/ haplotypes and IMF were expected based on the capacity of adiponectin to increase fatty acid oxidation in muscle cells (Yoon et al. (2006)) and on the presence of a QTL affecting marbling in the vicinity of the *ADIPOQ* gene in cattle (Morsci et al. (2006)). However, Morsci et al. (2006) and Shin and Chung (2013) were unable to find associations between *ADIPOQ* and marbling score in cattle, which is consistent with our findings.

## Conclusion

This study was performed to determine whether previously identified SNPs in the *ADIPOQ*, *ADIPOR1* and *ADIPOR2* genes are associated with different meat quality traits measured in the pig *longissimus* muscle. Associations were indeed found with meat color (Minolta L*, a* and b*), drip loss % and lactate dehydrogenase activity. However, these associations are breed specific with the mutant alleles segregating differently between DD, LL and YY pigs. This finding results in associations between meat quality traits and *ADIPOQ* SNPs and haplotypes that are observed in LL pigs only, and *ADIPOR2* SNPs and haplotypes showing associations with meat quality traits in DD and YY pigs. Our results demonstrate that selecting for specific *ADIPOQ* and *ADIPOR2* haplotypes may positively affect the *longissimus* meat colour, water holding capacity and glycolytic potential in pigs. However, these haplotypes are not associated with IMF content.

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