Novel SNPs of the porcine TRIP12 are associated with water holding capacity of meat

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ABSTRACT: Degradation of proteins during maturation of meat, mediated by the calpain/calpastatin system and the ubiquitination system, largely affects the tenderness and the water holding capacity (WHC) of meat. The thyroid hormone receptor interacting protein 12 (TRIP12) is known as a HECT domain-containing E3 ubiquitin-protein ligase that recognizes protein substrates for ubiquitination. This study aims to identify polymorphisms of the TRIP12 gene and to evaluate the relationship between genotype, transcript abundance, and meat quality traits in pigs. Two synonymous SNPs (XM_003484315.1:c.2211T>C, c.4957A>C) were identified that segregated among animals of herds of the breed German Landrace (DL, n = 269) and the commercial crossbreed of Pietrain × (German Large White × German Landrace) (PiF1, n = 300). Statistical analysis revealed associations between TRIP12 polymorphisms and the organismal traits related to water holding capacity, i.e. conductivity 45 min postmortem (CON₁, P < 0.1) and pH 24 h postmortem (pH₂₄, P < 0.1). Haplotype analysis revealed consistent effects on muscle CON₁ in the two populations (P < 0.1). Carriers of the minor alleles C at the two polymorphic sites tended to have higher transcript abundance as well as higher water holding capacity. The integrated analysis of genotypic and haplotypic variation, transcript abundance, and technological parameters of WHC indicates that the XM_003484315.1:c.2211T>C and c.4957A>C of TRIP12 are in linkage disequilibrium with a causal factor located in a cis-regulatory region, which affects in the first instance gene expression and in the second traits related to water holding capacity. Our results provide statistical-genetical evidence supporting TRIP12 as a functional candidate gene for water holding capacity of porcine M. longissimus dorsi.

Keywords: E3 ligase; swine; drip loss; expression; ubiquitination; protein degradation; meat quality

The ubiquitination system catalyzes a non-lysosomal proteolytic pathway. Upon activation of ubiquitin-activating enzyme E1 with ATP and transfer of ubiquitin to ubiquitin-conjugating enzymes (E2), ubiquitin-protein ligases (E3) bind the first ubiquitin molecule to target proteins. After polyubiquitination of the targets by E4 the tagged proteins become degraded by the 26S proteasome (Attaix et al., 2002; Taillandier et al., 2004; Herrera-Mendez et al., 2006). In muscle, the ubiquitination system contributes to the breakdown of myofibrillar and intermyofibrillar proteins as well as costameric connections including integrins. This process precedes postmortem during the shift from muscle to meat. The degree of postmortem protein degradation in muscle affects the water holding capacity (WHC) of meat (Huff-Lonergan and Lonergan, 2005). Reduced lateral shrinkage of myofibrils, due to breakdown of myofibrillar muscle proteins, increases WHC. However, in

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particular, the degradation of integrins leads to the formation of so-called drip channels in muscle decreasing the WHC of meat (Lawson, 2004; Zhang et al., 2006; Bee et al., 2007).

Thyroid hormone receptor interacting protein 12 (TRIP12) is the E3 enzyme and thus facilitates ubiquitination of substrates in conjunction with E1, E2, and E4 enzymes. TRIP12 contains a HECT domain and thus belongs to the HECT-domain E3 ubiquitin ligases, to which is common that a conserved cysteine residue contributes to the formation of an intermediate thioester bond with the ubiquitin C terminus before transfer of ubiquitin to the target proteins (Park et al., 2008, 2009; Rotin and Kumar, 2009; Pandya et al., 2010; Kim et al., 2011). In the recent studies, the expressions of genes encoding proteins of the ubiquitination system, including several E3 ligase genes, showed significant associations with the parameters of water holding capacity as well as tenderness of meat (Ponsuksili et al., 2008a,b; Ponsuksili et al., 2009, Damon et al., 2012). Therefore, the TRIP12 is considered as a functional gene for meat quality in our study, aiming to identify polymorphisms of TRIP12, to determine the mRNA expression in M. longissimus dorsi, and evaluate the three-way relationship of variation at the level of genotype, organismal traits related to meat characteristics, and transcript abundance in commercial pigs.

MATERIAL AND METHODS

Animals and phenotyping

Phenotypic data and genomic DNA of animals of the breed German Landrace (DL, n = 269), and the commercial crossbreed of Pietrain × (German Large White × German Landrace) (PiF1, n = 300) were used in this study. Animal care and tissue collection procedures followed the guidelines of the German Law of Animal Protection and the experimental protocols were approved by the Animal Care Committee of the Leibniz Institute of Farm Animal Biology (FBN). Carcass and meat quality traits were recorded according to the guidelines of German performance test (ZDS, 2004) after slaughter at the abattoir of the FBN. Technological meat traits that are indicators of water-holding capacity including drip loss (DRIP), pH, and conductivity 45 min postmortem (pH1, CON1) as well as at 24 h (pH24, CON24) were considered. Conductivity and pH-value were measured by Star-series equipment (Matthäus, Klausa, Germany) in M. longissimus dorsi between the 13th and the 14th rib. Drip loss was assessed by bag-method according to Honikel (1998). We collected and weighed size-standardized samples from the M. longissimus dorsi 24 h postmortem and re-weighed them after 24 h in a plastic bag at 4°C. In addition, meat to fat ratio (MFR) was included to the analysis as a comprehensive carcass trait.

SNP detection

DNA and RNA were isolated from M. longissimus dorsi tissue samples taken between the 13th and the 14th rib and snap frozen in liquid nitrogen immediately after slaughter. DNA was isolated by phenol-chloroform extraction. Total RNA was isolated with Tri-Reagent (Sigma-Aldrich, Taufkirchen, Germany). DNase (Roche, Mannheim, Germany) treatment and column based clean-up (Nucleospin RNA II kit; Macherey-Nagel, Düren, Germany). The quantity of RNA was determined on a NanoDrop ND-1000 spectrophotometer (PEQLAB, Erlangen, Germany), and RNA integrity was verified using 1% agarose gel. RNA (1.5 μg) was reverse transcribed to cDNA using Superscript III reverse transcriptase (Invitrogen, Karlsruhe, Germany) in a reaction containing 500 ng oligo (dT)11 VN primer and 500 ng random hexamer primer according to the manufacturer’s protocol.

The polymorphisms of TRIP12 were detected in cDNAs of 8 animals from different breeds (German Landrace, German Large White, Pietrain, and Duroc) by comparative sequencing of PCR fragments of each animal. The specific primers were designed according to the published sequences (GenBank Accession No. XM_003484315.1). The two pairs of primers were: FW1 5’-gttccaactgtcaactttag-3’ and RV1 5’-gcattacaccttcccttctg-3’; FW2 5’-gaaacatcccaacatggcttac-3’ and RV2 5’-catactgtagactctctttggtc-3’; amplicon sizes of the two fragments were 747 and 753 bp, respectively. Polymerase chain reactions (PCR) were performed in a 25 μl volume with 30 ng of cDNA, 1 X PCR buffer (with 1.5mM MgCl2), 200μM of each dNTP, 0.2μM of each primer, and 0.5 U of Taq DNA polymerase (GeneCraft, Münster, Germany). Cycling conditions were: initial denaturing at 95°C for 5 min; 35 cycles of 95°C for 30 s, 58°C for 30 s, and elongation at 72°C for 1 min; final extension...
at 72°C for 5 min. The PCR products were purified and comparatively sequenced in both directions on an ABI 3130 DNA Analyzer using ABI kits and protocols (Life Technologies, Darmstadt, Germany).

Genotyping

A total of 569 animals derived from the DL and PiF1 populations were genotyped at the novel SNPs by pyrosequencing. Genotyping primers were designed according to the published sequences (GenBank Accession No. NW_003537404.1) to amplify 160 bp and 295 bp fragments that contained the SNP c.2211T>C and c.4957A>C, respectively. The two pairs of primers were: PyFW1 5'-cagtgaacttatgccattgtacc-3' and PyRV1 5'-biotin-ctcaatgatccggtctca-3'; PyFW2 5'-tcttagttactgggtgttgtcttc-3' and PyRV2 5'-biotin-gatttgggtgatgtcagagaac-3'. The PCR procedure was: 35 cycles of 95°C for 30 s, 65°C for 30 s, 72°C for 1 min, and final extension at 72°C for 5 min. Washing and capture of templates as well as sequencing were performed using the Pyro Gold Reagent Kit (Qiagen, Hilden, Germany) on a PSQ 96MA Pyrosequencing instrument (Biotage, Uppsala, Sweden) according to the manufacturer's instructions as described before (Srikanthai et al., 2010).

Gene expression analyses

Transcript abundance of **TRIP12** was determined by quantitative real-time RT-PCR, qRT-PCR of ten animals per diplotype class of each of the DL and PiF1 population, in order to examine the relationship between variation of genotype and expression. According to the sequence (GenBank Accession No. XM_003484315.1), the specific primers of **TRIP12** gene for the qRT-PCR were designed as qPCRFW 5'-ccaacccagaaatcaaccagtc-3' and qPCRRV 5'-gatttccaacatggcccgggag-3', using a primer designing tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Housekeeping genes, ribosomal protein 32 (**RPL32**) (primers: forward: 5'-agcccaagatcgtcaaaaag-3', reverse: 5'-tggctcctccataacagtc-3') and hypoxanthine phosphoribosyltransferase 1 (**HPRT1**) (primers: forward: 5'-tgataagcttctcctctgtgta-3', reverse: 5'-tgagagatcctcctcacaattactgtca-3'), were considered as internal controls (Ponsuksili et al., 2008a). The reaction mixture contained 30 ng of cDNA, 0.5μM of forward and reverse primers, 5 μl of LightCycler SYBR Green 1 Master kit (Roche), and water to obtain a final volume of 10 μl. All of the samples were run in duplicate according to the protocol: initial denaturation of 95°C for 10 min, and 40 cycles of 95°C for 15 s, 10 s for annealing (60°C), and 15 s for elongation (72°C). The qRT-PCR analysis was performed in a BioRad iCycler iQV (BioRad Laboratories GmbH, Munich, Germany). All runs were checked for specificity by melting curve analysis and agarose gel electrophoresis of PCR products. For each assay, the serial dilutions of target PCR products were amplified and used to derive a standard curve to calculate the number of copies. Normalization for variation of RNA input was done by dividing the calculated mRNA copy numbers by a mean normalization factor derived from the expression of the housekeeping genes.

In order to address also the relationship of expression levels and traits related to the water holding capacity in commercial pigs, we obtained **TRIP12** expression levels from Affymetrix GeneChip Porcine Genome Array analyses of a subset of 173 individuals of the PiF1 animals (GEO Accession No. GSE322112) (Ponsuksili et al., 2012).

Statistical analyses

Allele and genotype frequencies at the SNPs were calculated separately for DL and PiF1, respectively, and the genotype distribution was tested for Hardy-Weinberg equilibrium by χ². Association between genotypic and phenotypic variation in the two commercial populations was analyzed using the MIXED procedure of SAS (Statistical Analysis System, Version 9.2, 2008):

\[ Y = \mu + \text{GENO} + \text{SEX} + \text{RYRI} + \text{sire} + \text{sd} + \text{SW} + e \]

where:
- \( Y \) = observation of traits
- \( \mu \) = overall mean
- GENO = genotype
- SEX = gender
- sd = slaughter date
- SW = slaughter weight
- e = residual error

Genotype, gender, and the genotype of **RYRI** (only in PiF1 population) were considered as fixed ef-
effects, whereas slaughter date and sire were random effects; slaughter weight was taken as a co-variable. The expectation-maximization (EM) algorithm (“haplotype” procedure of SAS) allowed assessing haplotypes segregating within both populations. The probability of each individual’s pair of haplotypes (diplotype) was assigned and only data of animals having diplotypes with the probability 1 were selected for further analysis. Subsequently, in order to refer to both SNPs at a time, a similar mixed model was applied where the diplotype, i.e. combination of haplotypes, was used as a fixed effect instead of genotype. Least Squares Means values for the genotypes and diplotypes were compared by the t-test, and P-values were adjusted by the Tukey-Kramer correction.

The association of gene expression levels obtained from microarray analyses with meat quality traits was evaluated using a MIXED procedure of SAS (Statistical Analysis System, Version 9.2, 2008) as follows:

\[
Y = \mu + \text{SEX} + \text{RYR1} + \text{sire} + \text{sd} + \text{SW} + \text{TRIP12exp} + e
\]

where:
- \(Y\) = observation of meat quality traits
- \(\mu\) = overall mean
- \(\text{SEX}\) = gender
- \(\text{sd}\) = slaughter date
- \(\text{SW}\) = slaughter weight
- \(e\) = residual error

Sex and the genotype of \text{RYR1} were considered as fixed effects, slaughter date (sd) and sire as random effects. Slaughter weight and gene expression (\text{TRIP12exp}) were included as co-variable for meat quality traits. The genotype-dependence of transcript abundance was analyzed by unpaired t-tests.

### RESULTS

In the current study, thyroid hormone receptor interacting protein 12 (\text{TRIP12}) is considered as a functional candidate gene for water holding capacity of meat due to its function as an E3 ubiquitin ligase, which is an important enzyme to tag proteins with a chain of ubiquitin molecules for subsequent degradation. The extent and the kinetics of postmortem protein degradation largely affect meat quality, in particular WHC.

### Table 1. Allele and haplotype frequencies of \text{TRIP12} gene in DL and PiF1 populations

<table>
<thead>
<tr>
<th>Polymorphic site</th>
<th>Allele/haplotype</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.2211T&gt;C</td>
<td>T</td>
<td>DL ((n = 269)) 0.555   PiF1 ((n = 300)) 0.726</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>DL ((n = 269)) 0.445   PiF1 ((n = 300)) 0.274</td>
</tr>
<tr>
<td>c.4957A&gt;C</td>
<td>A</td>
<td>DL ((n = 269)) 0.924   PiF1 ((n = 300)) 0.941</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>DL ((n = 269)) 0.076   PiF1 ((n = 300)) 0.059</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>DL ((n = 269)) 0.435   PiF1 ((n = 300)) 0.278</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>DL ((n = 269)) 0.482   PiF1 ((n = 300)) 0.661</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>DL ((n = 269)) 0.083   PiF1 ((n = 300)) 0.061</td>
</tr>
</tbody>
</table>

DL = German Landrace, PiF1 = Pietrain \times (German Large White \times German Landrace)

### Table 2. Least Squares Means (LSM) and standard errors (SE) for carcass and meat quality traits across genotypes of the \text{TRIP12} gene in DL and PiF1 populations

<table>
<thead>
<tr>
<th>Position</th>
<th>Traits</th>
<th>Populations</th>
<th>Genotype</th>
<th>Genotype LSM ± SE ((n))</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>CT</td>
<td>CC</td>
</tr>
<tr>
<td>c.2211T&gt;C</td>
<td>MFR</td>
<td>DL</td>
<td>0.51±0.01 (83)</td>
<td>0.47±0.01 (137)</td>
<td>0.51±0.02 (49)</td>
</tr>
<tr>
<td></td>
<td>CON(_1)</td>
<td>DL</td>
<td>4.65±0.11 (83)</td>
<td>4.29±0.09 (137)</td>
<td>4.21±0.13 (49)</td>
</tr>
<tr>
<td></td>
<td>CON(_1)</td>
<td>PiF1</td>
<td>5.28±0.19 (145)</td>
<td>4.75±0.22 (135)</td>
<td>4.55±0.44 (20)</td>
</tr>
<tr>
<td>c.4957A&gt;C</td>
<td>pH(_{24})</td>
<td>DL</td>
<td>5.48±0.01 (226)</td>
<td>5.51±0.02 (43)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>CON(_1)</td>
<td>PiF1</td>
<td>5.36±0.22 (271)</td>
<td>4.74±0.34 (29)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

DL = German Landrace, PiF1 = Pietrain \times (German Large White \times German Landrace), MFR = ratio of meat and fat area, CON\(_1\) = conductivity in M. longissimus dorsi between the 13\(^{th}\) and 14\(^{th}\) rib 45 min postmortem, pH\(_{24}\) = pH value in M. longissimus dorsi between the 13\(^{th}\) and 14\(^{th}\) rib 24 h postmortem within a row, means with different superscripts differ at \(^{ab}\)(\(P < 0.05\)), \(^{cd}\)(\(P < 0.1\))
Genotype and WHC

Two novel SNPs, XM_003484315.1:c.2211T>C and c.4957A>C, located within the HECT domain of TRIP12, were detected. Across both populations, the genotype distributions of the two silent SNPs were in Hardy-Weinberg equilibrium. In particular, the frequency of the minor allele C at locus c.4957A>C was low in both populations. Correspondingly, homozygous carriers of the minor allele C were detected at low frequency (< 1%) and were not considered for further analysis. To refer to both SNPs of TRIP12 at a time, three different haplotypes were derived segregating across the two populations: CA, TA, and TC. Among them, the frequency of haplotype TC was rare. The allele and haplotype frequencies of TRIP12 are shown in Table 1.

The associations of the two novel SNPs and their genotype combinations with traits related to WHC, in particular conductivity and pH, were evident. In the PiF1 population, the detected SNPs showed associations with muscle conductivity 45 min postmortem (CON1) at P = 0.05; at both SNPs the major alleles were associated with higher CON1. In the DL population, animals homozygous for the major alleles T at c.2211T>C and A at c.4957A>C exhibited higher CON1 and lower pH24 than other genotypes. However, the differences were only obvious at P = 0.09 (Table 2). Subsequently, haplotype analyses revealed consistent effects on CON1 across both populations, at P = 0.07 in DL and P = 0.06 in PiF1, with the most frequent diplotype TA/TA being associated with increased muscle conductivity (Table 3).

Table 3. Least Squares Means (LSM) and standard errors (SE) for meat and carcass traits across diplotypes of TRIP12 gene in pigs

<table>
<thead>
<tr>
<th>Traits</th>
<th>Populations</th>
<th>Diplotype</th>
<th>CA/CA</th>
<th>CA/TA</th>
<th>CA/TC</th>
<th>TA/TA</th>
<th>TA/TC</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFR</td>
<td>DL</td>
<td>0.51 ± 0.02 (47)</td>
<td>0.47 ± 0.01 (117)</td>
<td>0.45 ± 0.03 (22)</td>
<td>0.52 ± 0.01 (62)</td>
<td>0.48 ± 0.02 (21)</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>CON1</td>
<td>DL</td>
<td>4.21 ± 0.13 (47)</td>
<td>4.31 ± 0.09 (117)</td>
<td>4.19 ± 0.17 (22)</td>
<td>4.76 ± 0.12 (62)</td>
<td>4.32 ± 0.17 (21)</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>CON1</td>
<td>PiF1</td>
<td>4.59 ± 0.49 (17)</td>
<td>4.73 ± 0.23 (121)</td>
<td>4.96 ± 0.68 (15)</td>
<td>5.41 ± 0.21 (120)</td>
<td>4.26 ± 0.43 (27)</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

DL = German Landrace, PiF1 = Pietrain × (German Large White × German Landrace) crossbreed

Table 4. Relationship between TRIP12 polymorphisms and the transcript levels

<table>
<thead>
<tr>
<th>Genotype/diplotype</th>
<th>Transcript abundance (LSM ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DL</td>
</tr>
<tr>
<td>2211_TT</td>
<td>24 948.37 ± 1 673.65</td>
</tr>
<tr>
<td>2211_CT</td>
<td>26 789.61 ± 1 565.56</td>
</tr>
<tr>
<td>2211_CC</td>
<td>30 394.74 ± 1 476.02</td>
</tr>
<tr>
<td>4957_AA</td>
<td>27 077.14 ± 2 301.43</td>
</tr>
<tr>
<td>4957_AC</td>
<td>32 553.06 ± 2 217.72</td>
</tr>
<tr>
<td>Diploype CA/CA</td>
<td>32 981.43 ± 1 782.13</td>
</tr>
<tr>
<td>Diploype CA/TA</td>
<td>30 960.68 ± 1 756.02</td>
</tr>
<tr>
<td>Diploype CA/TC</td>
<td>28 560.85 ± 2 101.73</td>
</tr>
<tr>
<td>Diploype TA/TA</td>
<td>25 852.37 ± 1 820.15</td>
</tr>
<tr>
<td>Diploype TA/TC</td>
<td>27 859.14 ± 1 716.05</td>
</tr>
</tbody>
</table>

LSM = Least Squares Means, SE = standard errors, DL = German Landrace, PiF1 = Pietrain × (German Large White × German Landrace) crossbreed

\(^{a,b}\) for the same locus, within a column, means with different superscripts differ (P < 0.1)
Effects on carcass traits of TRIP12 polymorphisms were only evident in the DL for the allelic variation at c.2211T>C and also haplotypic variation (Tables 2 and 3). The animals with the haplotype combination TA/TA, of the major alleles, exhibited the highest Least Squares Means for meat to fat ration (P < 0.1).

Genotype and expression

To illustrate the existence of a causal polymorphism and its impact on the gene expression levels, we evaluated the relationship of TRIP12 polymorphisms and its transcript abundance using qRT-PCR. The statistical analysis of expression data derived from selected animals (ten animals per diplotype class of TRIP12 in the segregating populations) is shown in Table 4. Across all genotypes/diplotypes classes, the TRIP12 transcript abundances were similar among the DL and PiF1 populations. However, mean transcript abundances among various genotype/diplotypes groups in DL differed at P ≤ 0.1. In PiF1, only transcript abundance obtained in either homozygous or heterozygous animals at c.4957A>C differed at P = 0.06. The analysis revealed that pigs carrying the major alleles T and A at both loci had lower transcript abundance in both populations. Moreover, the effect of TRIP12 polymorphisms on the variation of gene expression was confirmed in the haplotype analysis across the two populations, with the diplotype TA/TA showing the lowest transcript abundance (Table 4).

Moreover, the relationship of transcript abundance and WHC was considered in PiF1. Transcript abundance detected by microarrays was compared with indicators of WHC. The TRIP12 transcript level was associated and negatively correlated with drip loss (P < 0.08; Pearson’s coefficient = –0.2).

DISCUSSION

Water holding capacity (WHC) of muscle cell depends on the capacity of proteins to bind water molecules and the structure of myofibrillar matrix to entrap the immobilized water (Huff-Lonergan and Lonergan, 2005). During rigor mortis, WHC of fresh pork is decreased due to the formation of purge loss that contains soluble components of the cells worsening nutritional and sensory properties of meat and at the same time increasing meat conductivity (Byrne et al., 2000; Lee et al., 2000; Fischer, 2007). The mechanism causing drip loss is explained by the combination of the shrinkage of muscle cells and the extent of filament proteins degradation. In the early postmortem period, in the presence of ATP, the ubiquitination process is activated in muscle cells. Subsequently, partial degrading costameric connections result in the reduced linkage of intermediate filament proteins in the muscle cell (Riley et al., 1988; Hilenski et al., 1992; Sekikawa et al., 2000, 2001). Moreover, integrins that link the cytoskeleton to the extracellular matrix, are also a target of the ubiquitin-proteasome system (Darom et al., 2010; Lobert and Stenmark, 2010). The degradation of integrins forms cracks between cell membrane that were termed drip channels. Thereby, the water is mobilized inside the myofibrillar matrix as well as expelled from the muscle cell throughout these drip channels, resulting in increased drip loss (Law-
Accordingly, holistic expression studies revealed an association of the expression of several genes encoding enzymes of the ubiquitination system with traits related to WHC (Ponsuksili et al., 2008a, b, 2009; Damon et al., 2012). Here we considered TRIP12, encoding a HECT domain E3 ubiquitin ligase, as a candidate gene that might affect the ubiquitination process and thereby meat quality. Mutations occurring within the HECT domain of E3 ligases may affect the ubiquitination process. Indeed, variation disrupting the formation of ubiquitin-thioester bonds between the conserved cysteine of the HECT and the ubiquitin C terminus disturb the initial substrate-ubiquitination event as well as the elongation of the polyubiquitin chain (Wang et al., 1999; Ogunjimi et al., 2010; Kajiro et al., 2011). In this study, the two synonymous SNPs were detected in the HECT domain; both do not cause a change of the structure of TRIP12. There was no evidence of any non-synonymous polymorphisms, i.e. in accordance with previous microarray experiments, any association of TRIP12 with WHC is likely to be due to variation in the transcript abundance. The SNPs detected here might be in linkage disequilibrium with polymorphism affecting the transcription level. Moreover, synonymous SNPs still might affect the function of gene due to altering the mRNA stability (Capon et al., 2004). Consequently, in order to get more insight into these possible scenarios, we addressed the relationships of genotype and WHC as well as genotype and expression here on the background of the already previously suggested association of E3 ubiquitin ligase expression levels and WHC. As illustrated in Figure 1, according to the association study, the animals carrying the minor alleles C at loci c.2211T>C and c.4957A>C, showed lower muscle conductivity and higher pH, which is indicative for higher WHC. At the same time, the minor alleles are associated with higher transcript abundance. The relationship between transcript abundance and WHC was evaluated and indicated a positive correlation which is in line with the relationship of gene variation and expression as well as gene variation and WHC (Figure 1). Thus, the integrated results of association and expression studies imply that the rare alleles C at both SNPs of the TRIP12 gene are in linkage disequilibrium with an allele of a causal site, which is most likely located in the cis-regulatory region of the TRIP12 gene and which increases the transcript abundance and decreases drip loss as well as muscle conductivity in the two commercial herds. Thus, selection for the minor alleles bears the potential of genetically improving meat quality in terms of WHC; however, the slight impact on meat to fat ratio observed in DL indicates that this might be on the cost of carcass quality. Also, any other association of TRIP12 with production and functional traits still needs to be evaluated.

CONCLUSION

The three-way relationship among TRIP12 polymorphisms, the transcript abundance, and water holding capacity suggests the existence of causal polymorphisms in cis-regulatory regions in incomplete linkage disequilibrium with the SNPs detected here. The putative causal polymorphism primarily affects the transcript abundance and, consequently, traits related to meat quality. Our study provides the statistical-genetical evidence to promote TRIP12 as a functional candidate gene for the water holding capacity of pork.

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EFSA, in cooperation with Member States, has decided to set up a database of external scientific experts able to assist its Scientific Committee, Scientific Panels, EFSA networks and respective working groups. EFSA will draw on this database to find experts to help deliver high-quality, independent and timely scientific advice.

You can be part of that team of top scientists helping EFSA support Europe’s decision makers in ensuring that Europe’s food is safe.

How can I apply?
Simply visit the EFSA website and fill in the form at www.efsa.europa.eu

Committed to ensuring that Europe’s food is safe.

European Food Safety Authority