

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

H.T.P. LOAN¹, E. MURANI¹, S. MAAK², S. PONSUKSILI³, K. WIMMERS¹

¹Institute for Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

²Institute for Muscle Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

³Research Group of Functional Genome Analysis, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

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 \times (German Large White \times 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 (CON₄₅, $P < 0.1$) 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

Supported by the German Research Foundation (Project No. WI 1754/10-14), German Academic Exchange Service (DAAD), Ministry of Education and Training of Vietnam, and Cantho University, Vietnam.

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 \times (German Large White \times 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, Klaus, 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'-gtccaactgtccaactttag-3' and RV1 5'-gcattacaccttccttctg-3'; FW2 5'-gaaacatcccaacatggcttac-3' and RV2 5'-catactgtagactctcttgggtc-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'-cagtgaacttatgcatgtttacc-3' and PyRV1 5'-biotin-ctcaatgatccggctgtcaa-3'; PyFW2 5'-tcttagttactgggtgtgtcttc-3' and PyRV2 5'-biotin-gattttggtgatgtcagagaacac-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'-ccaaccagaaatcaaccagtc-3' and qPCRRV 5'-gatttcaacatggcccgaggag-3', using a primer designing tool (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Housekeeping genes, ribosomal protein 32 (*RPL32*) (primers: forward: 5'-agcccaagatcgctcaaaaag-3', reverse: 5'-tgttgctccataaccaatg-3') and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) (primers: forward: 5'-gtgatagatccattcctatgactgtaga-3', reverse: 5'-tgagagatcatctccaccaattactt-3'), were considered as internal controls (Ponsuksili et al., 2008a). The

reaction mixture contained 30 ng of cDNA, 0.5 μM of forward and reverse primers, 5 μl of LightCycler SYBR Green I 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* *M. longissimus dorsi* 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 χ^2 . 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):

$$Y = \mu + \text{GENO} + \text{SEX} + \text{RYR1} + \text{sire} + \text{sd} + \text{SW} + e$$

where:

Y	= observation of traits
μ	= overall mean
GENO	= genotype
SEX	= gender
sd	= slaughter date
SW	= slaughter weight
e	= residual error

Genotype, gender, and the genotype of *RYR1* (only in PiF1 population) were considered as fixed ef-

fects, 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
 μ = overall mean
 SEX = gender
 sd = slaughter date
 SW = slaughter weight
e = residual error

Sex and the genotype of *RYR1* were considered as fixed effects, slaughter date (sd) and sire as ran-

Table 1. Allele and haplotype frequencies of *TRIP12* gene in DL and PiF1 populations

Polymorphic site	Allele/haplotype	Population	
		DL (<i>n</i> = 269)	PiF1 (<i>n</i> = 300)
<i>c.2211T>C</i>	<i>T</i>	0.555	0.726
	<i>C</i>	0.445	0.274
<i>c.4957A>C</i>	<i>A</i>	0.924	0.941
	<i>C</i>	0.076	0.059
	<i>CA</i>	0.435	0.278
Haplotype	<i>TA</i>	0.482	0.661
	<i>TC</i>	0.083	0.061

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

dom effects. Slaughter weight and gene expression (*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 (*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 2. Least Squares Means (LSM) and standard errors (SE) for carcass and meat quality traits across genotypes of the *TRIP12* gene in DL and PiF1 populations

Position	Traits	Populations	Genotype LSM ± SE (<i>n</i>)			<i>P</i> -value
			<i>TT</i>	<i>CT</i>	<i>CC</i>	
<i>c.2211T>C</i>	MFR	DL	0.51 ^a ± 0.01 (83)	0.47 ^b ± 0.01 (137)	0.51 ^a ± 0.02 (49)	0.04
	CON ₁	DL	4.65 ^c ± 0.11 (83)	4.29 ^d ± 0.09 (137)	4.21 ^d ± 0.13 (49)	0.09
	CON ₁	PiF1	5.28 ^c ± 0.19 (145)	4.75 ^d ± 0.22 (135)	4.55 ^d ± 0.44 (20)	0.05
<i>c.4957A>C</i>			<i>AA</i>	<i>AC</i>		
	pH ₂₄	DL	5.48 ^c ± 0.01 (226)	5.51 ^d ± 0.02 (43)		0.08
	CON ₁	PiF1	5.36 ^a ± 0.22 (271)	4.74 ^b ± 0.34 (29)		0.04

DL = German Landrace, PiF1 = Pietrain × (German Large White × German Landrace), MFR = ratio of meat and fat area, CON₁ = conductivity in *M. longissimus dorsi* between the 13th and 14th rib 45 min postmortem, pH₂₄ = pH value in *M. longissimus dorsi* between the 13th and 14th rib 24 h postmortem

within a row, means with different superscripts differ at ^{a,b}(*P* < 0.05), ^{c,d}(*P* < 0.1)

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

Traits	Populations	Diplotype LSM \pm SE (<i>n</i>)					<i>P</i> -value
		CA/CA	CA/TA	CA/TC	TA/TA	TA/TC	
MFR	DL	0.51 ^b \pm 0.02 (47)	0.47 ^a \pm 0.01 (117)	0.45 ^a \pm 0.03 (22)	0.52 ^b \pm 0.01 (62)	0.48 ^{ab} \pm 0.02(21)	0.06
CON ₁	DL	4.21 ^a \pm 0.13 (47)	4.31 ^a \pm 0.09 (117)	4.19 ^a \pm 0.17 (22)	4.76 ^b \pm 0.12 (62)	4.32 ^a \pm 0.17(21)	0.07
CON ₁	PiF1	4.59 ^a \pm 0.49 (17)	4.73 ^a \pm 0.23 (121)	4.96 ^{ab} \pm 0.68 (15)	5.41 ^b \pm 0.21 (120)	4.26 ^a \pm 0.43(27)	0.06

DL = German Landrace, PiF1 = Pietrain \times (German Large White \times German Landrace), MFR = ratio of meat and fat area, CON₁ = conductivity in *M. longissimus dorsi* between the 13th and 14th rib 45 min postmortem

^{a,b} within a row, means with different superscripts differ ($P < 0.1$)

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 4. Relationship between *TRIP12* polymorphisms and the transcript levels

Genotype/diplotype	Transcript abundance (LSM \pm SE)	
	DL	PiF1
2211_ TT	24 948.37 ^a \pm 1 673.65	27 422.35 \pm 1 954.43
2211_ CT	26 789.61 ^{ab} \pm 1 565.56	29 439.17 \pm 2 118.67
2211_ CC	30 394.74 ^b \pm 1 476.02	33 075.02 \pm 1 845.38
4957_ AA	27 077.14 ^a \pm 2 301.43	29 796.73 ^b \pm 2 313.04
4957_ AC	32 553.06 ^b \pm 2 217.72	37 072.23 ^a \pm 2 958.37
Diplotype CA/CA	32 981.43 ^b \pm 1 782.13	34 082.54 \pm 2 695.42
Diplotype CA/TA	30 960.68 ^{ab} \pm 1 756.02	33 913.70 \pm 2 584.38
Diplotype CA/TC	28 560.85 ^{ab} \pm 2 101.73	30 557.87 \pm 2 068.27
Diplotype TA/TA	25 852.37 ^a \pm 1 820.15	27 755.69 \pm 2 410.86
Diplotype TA/TC	27 859.14 ^{ab} \pm 1 716.05	29 439.18 \pm 2 543.76

LSM = Least Squares Means, SE = standard errors, DL = German Landrace, PiF1 = Pietrain \times (German Large White \times 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/diplotype groups in DL differed at $P \leq 0.1$. 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-

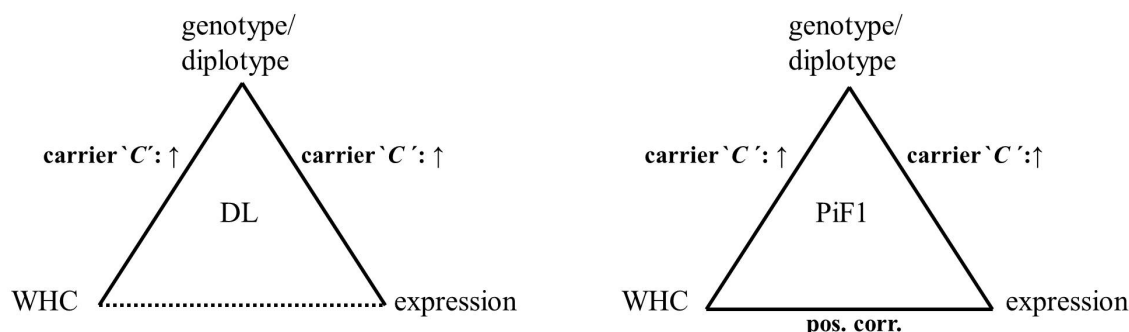


Figure 1. Three-way relationship of *TRIP12* polymorphism, transcript abundance, and water holding capacity (WHC) in commercial pigs represented by the triangle-legs. Solid lines represent relationships that were experimentally shown as indicated alongside; dotted line indicates suggested relationships

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

son, 2004; Zhang et al., 2006; Bee et al., 2007). 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 abun-

dance 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.

Acknowledgement

Thanks are due to the Pig Breeding Association of Mecklenburg-Western Pommerania.

REFERENCES

- Attaix D., Combaret L., Pouch M.-N., Taillandier D. (2002): Cellular control of ubiquitin-proteasome-dependent proteolysis. *Journal of Animal Science*, 80, E56–E63.
- Bee G., Anderson A.L., Lonergan S.M., Huff-Lonergan E. (2007): Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. *Meat Science*, 76, 359–365.
- Byrne C.E., Troy D.J., Buckley D.J. (2000): Postmortem changes in muscle electrical properties of bovine *M. longissimus dorsi* and their relationship to meat quality attributes and pH fall. *Meat Science*, 54, 23–34.
- Capon F., Allen M.H., Ameen M., Barker J.N., Trembath R.C. (2004): A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. *Human Molecular Genetics*, 13, 2361–2368.
- Damon M., Wyszynska-Koko J., Vincent A. (2012): Comparison of muscle transcriptome between pigs with

- divergent meat quality phenotypes identifies genes related to muscle metabolism and structure. PLoS ONE, 7, 33763–33777.
- Darom A., Bening-Abu-Shach U., Broday L. (2010): RNF-121 is an endoplasmic reticulum-membrane E3 ubiquitin ligase involved in the regulation of β -integrin. Molecular Biology of the Cell, 21, 1788–1798.
- Fischer K. (2007): Drip loss in pork: influencing factors and relation to further meat quality traits. Journal of Animal Breeding and Genetics, 124, 12–18.
- Herrera-Mendez C.H., Becila S., Boudjellal A., Ouali A. (2006): Meat ageing: reconsideration of the current concept. Trends in Food Science and Technology, 17, 394–405.
- Hilenski L.L., Terracio L., Haas A.L., Borg T.K. (1992): Immunolocalization of ubiquitin conjugates at Z-bands and intercalated discs of rat cardiomyocytes *in vitro* and *in vivo*. Journal of Histochemistry and Cytochemistry, 40, 1037–1042.
- Honikel K. (1998): Reference methods for the assessment of physical characteristics of meat. Meat Science, 49, 447–457.
- Huff-Lonergan E., Lonergan S.M. (2005): Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. Meat Science, 71, 194–204.
- Kajiro M., Tsuchiya M., Kawabe Y.-I., Furumai R., Iwasaki N. (2011): The E3 ubiquitin ligase activity of *TRIP12* is essential for mouse embryogenesis. PLoS ONE, 6, 25871–25879.
- Kim H.C., Steffen A.M., Oldham M.L., Chen J., Huibregtse J.M. (2011): Structure and function of a HECT domain ubiquitin-binding site. EMBO reports, 12, 334–341.
- Lawson M.A. (2004): The role of integrin degradation in post-mortem drip loss in pork. Meat Science, 68, 559–566.
- Lee S., Norman J.M., Gunasekaran S., van Laack R.L.J.M., Kim B.C., Kauffman R.G. (2000): Use of electrical conductivity to predict water-holding capacity in post-rigor pork. Meat Science, 55, 385–389.
- Lobert V.H., Stenmark H. (2010): Ubiquitination of α -integrin cytoplasmic tails. Communicative and Integrative Biology, 3, 583–585.
- Ogunjimi A.A., Wiesner S., Briant D.J., Varelas X., Sicheri F., Forman-Kay J., Wrana J.L. (2010): The ubiquitin binding region of the *Smurf* HECT domain facilitates polyubiquitylation and binding of ubiquitylated substrates. The Journal of Biological Chemistry, 285, 6308–6315.
- Pandya R.K., Partridge J.R., Love K.R., Schwartz T.U., Ploegh H.L. (2010): A structural element within the *HUWE1* HECT domain modulates self-ubiquitination and substrate ubiquitination activities. The Journal of Biological Chemistry, 285, 5664–5673.
- Park Y., Yoon S.K., Yoon J.-B. (2008): *TRIP12* functions as an E3 ubiquitin ligase of *APP-BP1*. Biochemical and Biophysical Research Communications, 374, 294–298.
- Park Y., Yoon S.K., Yoon J.-B. (2009): The HECT domain of *TRIP12* ubiquitinates substrates of the ubiquitin fusion degradation pathway. The Journal of Biological Chemistry, 284, 1540–1549.
- Ponsuksili S., Jonas E., Murani E., Phatsara C., Srikanthai T., Walz C. (2008a): Trait correlated expression combined with expression QTL analysis reveals biological pathways and candidate genes affecting water holding capacity of muscle. BMC Genomics, 9, 367.
- Ponsuksili S., Murani E., Phatsara C., Jonas E., Walz C., Schwerin M. (2008b): Expression profiling of muscle reveals transcripts differentially expressed in muscle that affect water-holding capacity of pork. Journal of Agriculture and Food Chemistry, 56, 10311–10317.
- Ponsuksili S., Murani E., Phatsara C., Schwerin M., Schellander K., Wimmers K. (2009): Porcine muscle sensory attributes associate with major changes in gene networks involving *CAPZB*, *ANKRD1*, and *CTBP2*. Functional and Integrative Genomics, 9, 455–471.
- Ponsuksili S., Du Y., Murani E., Schwerin M., Wimmers K. (2012): Elucidating molecular networks that either affect or respond to plasma cortisol concentration in target tissues of liver and muscle. Genetics, 192, 1109–1122.
- Riley D.A., Bain J.L., Ellis S., Haas A.L. (1988): Quantitation and immunocytochemical localization of ubiquitin conjugates within rat red and white skeletal muscles. Journal of Histochemistry and Cytochemistry, 36, 621–632.
- Rotin D., Kumar S. (2009): Physiological functions of the HECT family of ubiquitin ligases. Molecular Cell Biology, 10, 398–409.
- Sekikawa M., Shimada K., Fukushima M., Ishikawa T., Wakamatsu J., Mikami M. (2000): Presence of ubiquitin in bovine post-mortem cardiac muscle. Food Chemistry, 69, 315–318.
- Sekikawa M., Yamamoto M., Fukushima M., Shimada K., Ishikawa T., Mikami M. (2001): Effect of proteasome inhibitor on sarcoplasmic protein of bovine skeletal muscle during storage. Food Chemistry, 73, 17–21.
- Srikanthai T., Murani E., Wimmers K., Ponsuksili S. (2010): Four loci differentially expressed in muscle tissue depending on water-holding capacity are associated with meat quality in commercial pig herds. Molecular Biology Reports, 37, 595–601.
- Taillandier D., Combaret L., Pouch M.-N., Samuels S.E., Béchet D., Attaix D. (2004): The role of ubiquitin-proteasome-dependent proteolysis in the remodelling of skeletal muscle. Proceedings of the Nutrition Society, 63, 357–361.
- Wang G., Yang J., Huibregtse J.M. (1999): Functional domains of the Rsp5 ubiquitin-protein ligase. Molecular and Cellular Biology, 19, 342–352.
- ZDS (2004): Richtlinie für die Stationsprüfung auf Mastleistung, Schlachtkörperwert und Fleischbeschaffenheit

beim Schwein. Zentral Verband der Deutschen Schweineproduktion eV. Ausschuss fuer Leistungsprüfung und Zuchtwertschaetzung, Bonn, Germany.

Zhang W.G., Lonergan S.M., Gardner M.A., Huff-Lonergan E. (2006): Contribution of postmortem changes of inte-

grin, desmin and μ -calpain to variation in water holding capacity of pork. Meat Science, 74, 578–585.

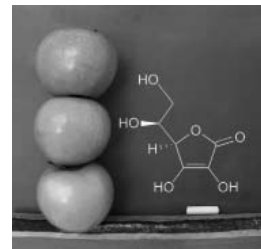
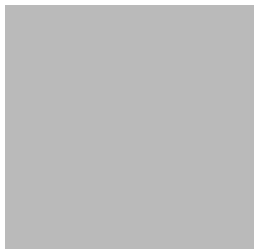
Received: 2013–03–25

Accepted after corrections: 2013–08–15

Corresponding Author

Prof. Dr. Klaus Wimmers, Leibniz Institute for Farm Animal Biology, Institute for Genome Biology,
Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany

Tel. +493 820 868 700, fax +493 820 868 702, e-mail: wimmers@fhn-dummerstorf.de



Sign up and be an EFSA expert

- Want to make a difference to EU food safety?
- Contribute to EU risk assessment?
- Value high profile networking with peers?
- Driven by excellence?

EFSA invites leading scientists to sign up to its new expert database.

EFSA is the European Union's scientific risk assessment body on food and feed safety, nutrition, animal health and welfare, plant health and protection.

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